XVIth European Symposium on the Quality of Poultry Meat
&
Xth European Symposium on the Quality of Eggs and Egg Products

September 23rd – 26th, 2003

Saint-Brieuc
France

Proceedings

Volume I : New European regulations and their impact - Hygiene and microbiology of poultry production - Evaluation and control of risks in poultry production

Volume I : New European regulations and their impact - Hygiene and microbiology of poultry production - Evaluation and control of risks in poultry production
Volume II : Quality of Poultry Meat
Volume III : Quality of Eggs and Egg Products

Realisation of proceedings : Geneviève Clément and Marie-Pierre Rouxel, ISPAIA

Introduction

It is our pleasure to present the proceedings of the Xth European Symposium on the Quality of Eggs and Egg Products & XVIth European Symposium on the Quality of Poultry Meat which is being held in Saint Brieuc – Ploufragan, France.

The scientific programme of the symposium has been prepared from papers by invited speakers, oral presentations and posters. Efforts have been made to ensure that program for the symposium will effectively cover all aspects of egg and meat quality. The symposium consists of a total twelve sessions. The symposia are organized under auspices of the French branch of the WPSA, the poultry meat and egg quality working groups with the collaboration of AFSSA (French Agency for Food Safety), INRA (French Institute for Agronomical Research), ISPAIA (Higher Institute for Animal Production and Food Industries) and ITAVI (Technical Institute for Poultry Production). We are extending our thanks to all scientific committee members and reviewers of manuscripts for their contribution.

The proceedings have been published in three volumes prior to the symposium. Volumes 1, 2 and 3 contain the sessions on health, meat and egg quality respectively.
This first volume concerns the evolution of the European regulation and most of the problems of food safety identified as potentially associated with the consumption of poultry products in year 2003. If *Salmonella* is still a subject of concern for the producers and the industry, some solutions to eradicate the contamination will be proposed during this symposium. Emerging problems like *Campylobacter* contamination or presence of residues in the poultry products are new preoccupying subjects of concern and will be approached during this week. We hope that this symposium will be a good opportunity to underline the progress that were done in the field of poultry products food safety and to evaluate what has still to be done.
We wish you a successful symposium and an enjoyable stay in France.

Gilles Salvat	Yves Nys	E. Baéza
AFSSA-WPSA	INRA-WPSA	INRA-WPSA

Introduction

Nous avons l'honneur et le plaisir de vous présenter les actes du Xème Symposium Européen sur la Qualité des Oeufs et des Ovoproduits & du XVIème Symposium Européen sur la Qualité des Viandes de Volailles qui se tiendront à Saint Brieuc – Ploufragan, France.

Le programme scientifique du symposium a été préparé avec les textes des orateurs invités, des présentations orales et des posters. Nous nous sommes assurés que le programme du symposium couvrirait tous les aspects de la qualité des viandes de volailles et des œufs. Le symposium comporte douze sessions. Les symposiums sont organisés grâce à l'aide de la branche française de la WPSA, les groupes de travail sur la qualité des oeufs et de la viande de volailles en collaboration avec l'AFSSA (Agence Française de Sécurité Sanitaire des Aliments), l'INRA (Institut National de Recherche Agronomique), l'ISPAIA (Institut Supérieur des Productions Animales et de l'Alimentation) et l'ITAVI (Institut Technique de l'Aviculture). Nous remercions tous les membres du comité scientifique ainsi que les lecteurs des manuscrits pour leur aide dans la préparation du programme et des actes.

Les actes comportent trois volumes. Les volumes 1, 2 et 3 contiennent respectivement les sessions sur la qualité sanitaire, la qualité de la viande et la qualité des oeufs.

Ce volume 1 qui s'intéresse à l'évolution de la réglementation sanitaire européenne et aux problèmes de qualité sanitaires des viandes de volailles et des œufs se veut le reflet des préoccupations de la filière avicole en 2003. Si les Salmonelles occupent encore une large part dans les préoccupations de sécurité sanitaire des aliments des intervenants de la filière, les solutions en matière de lutte contre ces contaminants sont largement développées dans ce recueil. L'émergence des préoccupations en matière de contamination des produits par *Campylobacter* ou par les résidus potentiellement toxiques de différentes origines est sensible au travers des propositions de communications et des choix du comité de lecture. Gageons que ce symposium soit l'occasion de rappeler combien la sécurité des produits de volailles a progressé et qu'il nous permette de mesurer l'étendu du champ d'investigation qu'il nous reste à défricher.

Nous espérons que vous serez intéressés par ce symposium et nous vous souhaitons un agréable séjour en France.

Gilles Salvat	Yves Nys	E. Baéza
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Session 1
New European regulations and their impact

EGG PRODUCTION AND TRADE PRESENT AND PERSPECTIVES

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Chief Basic Data Branch, FAO, Rome, Italy

Keywords: Egg, Poultry, Production, Statistics and Chicken.

Abstract

From the early 1960s to 2000-2002 world eggs production went up 268 %: 898 % in developing countries and nearly 61 % in developed countries. Developing countries production accounts now for 67 % of world production, against 25 %in the early 1960s.
At world level from early 1960s to early 1980s hen eggs production per laying hen went up 12.5 %from 7.2 to 8.1 kg. From early 1980s to 2000-2002, 24.7 % from 8.1 to 10.1 Kg.
This is due to the changes that have taken place over the last 25 years in many countries, particularly with regard to the growth of a modern specialized and intensive sector alongside the traditional sector.

Résumé

À partir du début des années 60 jusqu'en 2000-2002, la production mondiale d'œufs a augmenté de 268% : 898% dans les pays en voie de développement et de presque 61% pour les pays développés. La production des pays en voie de développement représente 67% de la production mondiale, contre 25% au début des années 60. Au niveau mondial, à partir du début des années 60 jusqu'au début des années 80, la production d'œufs de poule par poule couveuse a augmentée de 12,5% de 7,2 Kg à 8,1 Kg. À partir du début des années 80 jusqu'aux années 2001-2002 de 8,1 Kg à 10,1 Kg. Ceci est dû aux changements qui se sont effectués ces dernières 25 années, dans beaucoup de pays, en particulier en ce qui concerne la croissance d'un secteur moderne, spécialisé et intensif à côté d'un secteur traditionnel.

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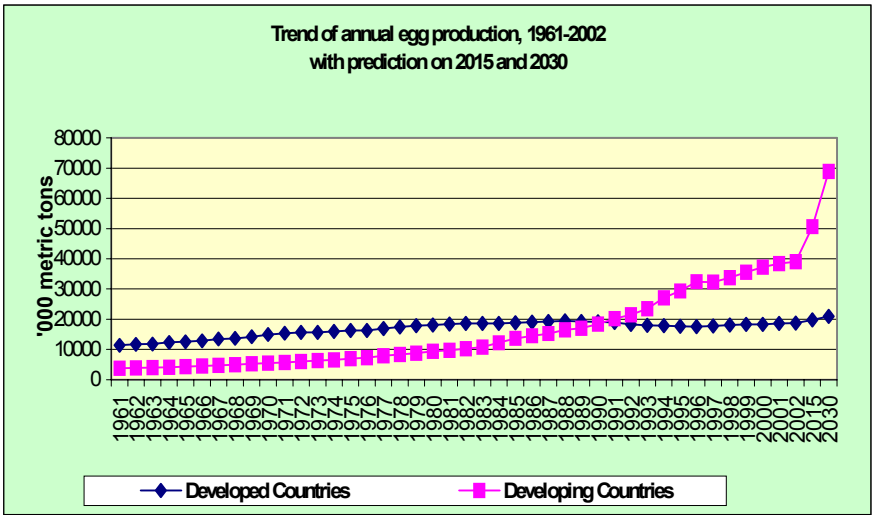
Egg production- Current situation and the future forecast

Using FAO's statistics, between 1961 and 2002, annual world egg production rose almost 4 times to reach about 57.8 million tons, of which 53.5 million are hen eggs (about 6 % are hatching eggs). During this period, the rates of increase recorded for eggs production were, at least at the world level, quite high and costant as per the table reported below.

Rates of increase by continent (%)					
	1961/1965	**1971/1975**	**1981/1985**	**1991/1995**	**1998/2002**
World	**112**	**110**	**115**	**120**	**112**
Africa	**121**	**125**	**119**	**110**	**113**
Asia	**123**	**114**	**143**	**151**	**115**
Europe	**109**	**114**	**103**	**83**	**103**
North&C. America	**107**	**96**	**101**	**107**	**113**
Oceania	**106**	**103**	**90**	**88**	**99**
South America	**109**	**122**	**119**	**112**	**112**

Production is further predicted to increase another 36% by the year 2015 and further increasing 27% by 2030. The increases are due to the rapid expansion in egg production of developing countries.

Starting from a low base of 3.8 million tons in 1961, developing countries achieved an increase of egg production almost 10-fold, with growth mainly in Asia. This trend was



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seriously influenced by the development of egg production in mainland China, from 1.5 million MT in 1961 to 23.8 million MT in 2002, with an annual growth rate of about 8 percent. The growth is predicted to keep increasing in China for another 30 years. In the developing countries, egg production will increase 50% by the year 2015 and 36% by year 2030. The egg production in mainland China is forecast to increase 29% by 2015 (26.1 million MT) and 19% by 2030 (31.2 million MT). On the other hand, developed countries production has remained flat. In term of absolute figures, annual egg production of developed countries has remained around 18 million MT for the last decade.

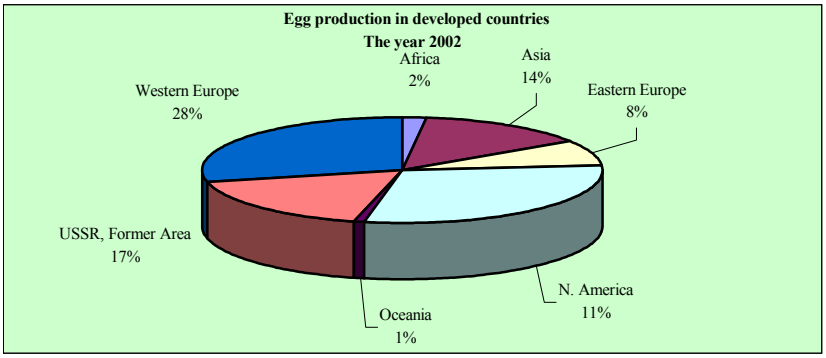
By the year 2015 and year 2030, the developed countries egg production is going to increase only 10% and 6% respectively.

In the year 1990 the contribution of developed and developing countries to the world egg production was approximately equal (49.1% developing countries and 50.9% developed countries).

But by this year developed countries production represented less than a third of total world egg production, dropping from about half in 1990s.

Furthermore, by the year 2015 egg production of developed countries will represent 28% of the world egg production and it is expected to drop to 23% by 2030.

Looking into the future, for the developed countries, 55% of the production will be from Europe and North American and it will stay about the same going up to the year 2030.



Out of the total quantity of eggs produced by developed countries in the year 2002 (18.8 million MT), 41% of production came from Europe and North America; countries of the former Soviet Union contributed 17% of the global production figures.

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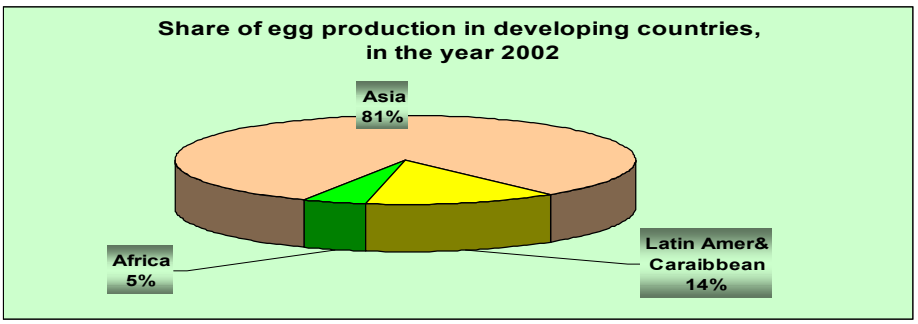

In the same year, Asian countries alone contributed 81% of total egg production of developing countries. In fact, egg production of Asian developing countries (32 million MT) was almost double that produced in developed countries in 2002.

Asian countries will contribute less than two thirds (2/3) of the total developing countries egg production by year 2015, it is a decrease comparing to current level, and it will keep decreasing its shares to around half by 2030. As for the share of the total world egg production, Asian countries are forecasted to contribute 42% by 2015 and down to 39% by 2030. The **continental** breakdown over the last 12 years shows the spectacular change of egg production in Asia (240.2%). In North & Central America, Africa and South America egg production in 2002 against 1990 was 136%, 138 and 131% respectively, which could be considered as a moderate growth rate. During the same period, total egg production in Europe recorded a negative trend.

Eggs primary Production (-000Mt)	**Year**				**Production change 2002/1990 %**
	1990	**1995**	**2000**	**2002**	
Asia	**14507.0**	**26383.8**	**32382.3**	**34849.3**	**240.2**
Africa	**1556.0**	**1775.5**	**1977.2**	**2146.8**	**138.0**
Europe	**11125.0**	**9595.3**	**9506.1**	**9772.7**	**87.8**
North & C. America	**5779.5**	**6411.5**	**7467.4**	**7882.5**	**136.4**
Oceania	**245.8**	**210.4**	**266.9**	**206.8**	**84.1**
South America	**2253.4**	**2655.7**	**2728.1**	**2945.5**	**130.7**
World	**37554.0**	**47032.7**	**54328.0**	**57803.7**	**153.9**

Forecasting continental production, one sees significant increases in South America and Africa by year 2030 (213% and 259% growth, respectively). Meanwhile, North & Central America will increase producing too. But Europe production is expected grow only slowly. This trend is caused by the change in consumer demand. Per capita egg

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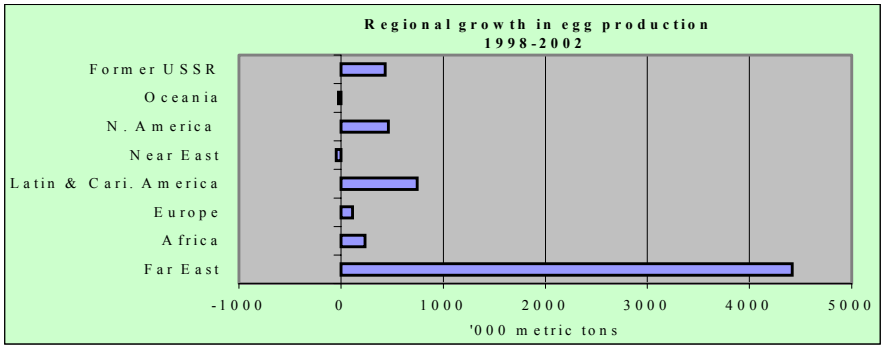
consumption, which is very high in Europe (12.1 kg per year), is problably at its maximum level.

Continental breakdown of egg production prediction in 2015 and 2030 is as follows.

	Eggs Primary Production (000Mt)		Production Change (%)	
	2015	2030	2015/2002	2030/2002
Asia	43364.6	56620.4	124	162
Africa	3206.1	5128.8	149	239
Europe	10635.9	11222.9	109	115
North & Central America	8764.4	10738	111	136
South America	4130.9	5822.3	140	198
World	70442.9	89941.7	122	156

In the period 1990-2002, the major contributors to the growth of annual egg production were: mainland China (+15.9 million MT - 2 million of this was duck eggs), United States of America (+1 million MT), Mexico (+ 0.9 million MT), India (+0.8 million MT), Brazil (+0.3 MT) and Turkey (+0.1 million MT). The share of output produced by these countries represented about 60% of total world egg production in the year 2002.

The contribution of these first ten producers to the increase in world egg production represented 96.3% of total growth; China (83% of world egg production growth) being the absolute leader for this period (1992-2002).



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90		2002		
Country	Production (thousand metric tons)	Country	Production (thousand metric tons)	Production growth (1990-2002) (thousand metric tons)
China mainland	7946	China mainland	23799	+15853
USA	4034	USA	5128	+1094
Russia	2641	Japan	2535	+116
Japan	2419	Russia	2081.5	-559.5
India	1161	India	2010	+849
Brazil	1256.3	Mexico	1885.1	+875.3
Mexico	1009.8	Brazil	1595	+338.7
Germany	985	France	1010	+123.2
Ukraine	944	Germany	886.6	-98.4
France	886.8	Turkey	530	+145
Total	23282.9	Total	41460.2	+19855.4
Share of world production	62	Share of world production	71.7%	**98%**

Only in the last four years (1998-2002), egg production in the Far East increased by 4.4 million MT, this evolution being seriously influenced by the egg quantities produced in China, India, Iran, Myanmar and Indonesia. In North America, the production growth was influenced by the trend recorded above all in Mexico.
On the other hand in some countries as France, Spain, Greece Switzerland, Moldova, Czech Republic, Belarus, and Finland, egg production recorded a negative trend in recent years.

Eggs Production Forecast (000Mt)

Country/Year	2015	2030	Change
EU15	5392.8	5239.6	-53.2

Note: This forecast was based on estimated current levels of which have come in much lower than expected.

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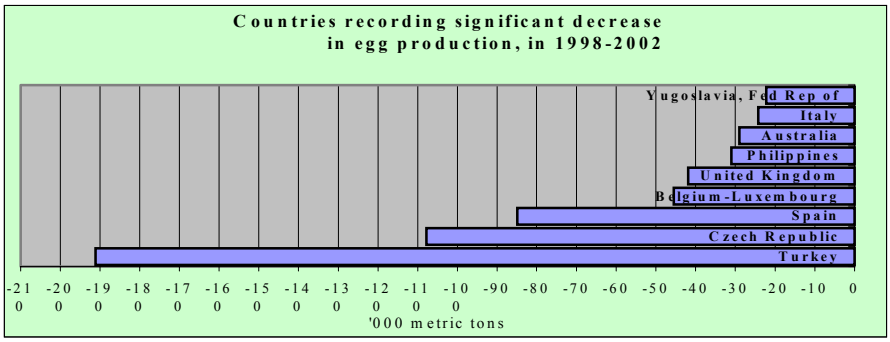
More on the forecast of egg production for the next 30 years



So, according to our studies world egg production is forecast to reach 70 million MT by 2015 and 90 million MT by 2030. The factors that will allow this consistent increase in eggs production to be reached are population growth, increase of real incomes which will improve the population's level of life and increase the egg market. The level in egg price will contribute too. On the other hand, the development estimated for the future could be interrupted by an economic or political crisis, or by more technical factors such as high feed prices.

For example, the decline in Turkish eggs production, shown in the previous table could be due to the crisis which hit the poultry and egg sector as a consequence of the more general economic crisis which affected the country during the last three years. This caused reduced incomes which have lowered consumer spending.

Most of the increase will originate in the **developing world**.

Production in **developed countries** is expected to increase from current levels of 19 million MT to only 20 million MT in the next 15 years and only another 2 million MT by 2030. In many **developed countries**, food saturation or over-consumption has meant that growth has halted. While production in developing countries based on revised growth rates, may reach at least 50 million metric tons – making a world total of 70 million tons in the year 2015 **- 22% above current production**. Continuing on to 2030, developing country's production will go up to 70 million MT mark and make the world total up to 90 million MT, 56% above current production!

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Estimated egg production increase, The Year 2030 Base year: 2001	
	-000 Metric tons
China	+ 8 204
Mexico	+ 1 590
India	+ 5 356
Brazil	+ 1 576
World	+ 33 348

Between the year 2001 and 2030 China's egg production will increase more than a third (still this depends on the availability of feed and feed prices). Together with the increase forecast for **India** this will minimize the remarkable performance estimated for Brazil and Mexico.

Between 2015 and 2030 expected growth will be: China (+5068 thousand MT), United States (+638.3 thousand MT), India (+2925.1 thousand MT), Brazil (+929.2 thousand MT), Mexico (+989.1 thousand MT), and Russia (+252.9 thousand MT). The European Union countries, however, have negative growth from 2015 to 2030 (-153.2 thousand MT) and Japan (-17.9 thousand MT) as well.

2015		2030		Predicted **Production** growth (2030-2015) (000Mt)
Country	Production (000Mt)	Country	Production (000Mt)	
China	26097.6	China	31166	5068.4
USA	5203.2	USA	5841.5	638.3
Russia	2094.6	Russia	2347.5	252.9
Japan	2937.5	Japan	2919.6	-17.9
India	4336.2	India	7261.3	2925.1
Brazil	2229.2	Brazil	3158.4	929.2
Mexico	2483	Mexico	3472.1	989.1
Ukraine	563.1	Ukraine	625.6	62.5
EU15	5392.8	EU15	5239.6	-153.2
Total Share of the World Production	51337.2 72.9%	**Total Share of the World Production**	62031.6 69%	10694.4

There are potential areas of growth in some African countries, (given income growth and an end to civil strife). Even greater potential exists in Central and South America (the requirement once again being prosperity). In these regions there is a large market particularly in Chile and Mexico and to a lesser extent Costa Rica, Colombia and

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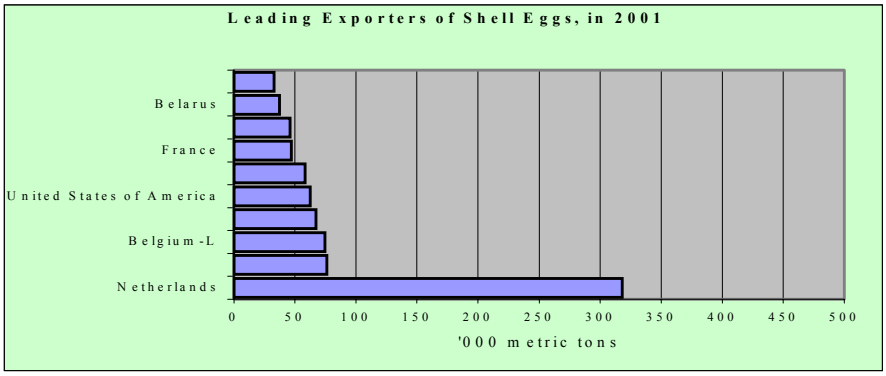
Venezuela.
By year 2030, according to the FAO forecast, Mexico is still a great egg demand country along with Colombia, and to a lesser extent Venezuela, Chile and Costa Rica. In the Middle East Iran will continue to show strong consumer demand. Iran is now self-sufficient in eggs and exporting around $0.5million, plus $11 million of egg pasta (macaroni) in 2001 (down from $55 million in 1997 and $25 million in 1999). Iran will continue to be a self-sufficient country in egg industry. Iran is the third largest wheat importer in the world, and also imports considerable quantities of animal feed.
The wheat and eggs are transformed into egg pasta (macaroni) which is exported to its neighbors.
The future will see continued production and consumption growth in India and its egg surplus is expected to dry up for next 30 years, as well as in the large Asian populous countries of Pakistan, Bangladesh, Indonesia, the Philippines, and Turkey. Note that Indonesia will be faced negative supply surplus already by 2015; while Bangladesh will have the positive increase in supply surplus in the next 30 years.
The oil producers, of course, with cheap energy, will continue to be growth areas.
In North Africa, Egypt and Libya, the market expansion is expected but not significant though the net trade is increasing for the next 30 years; while in the Middle East, Saudi Arabia's market is expected to expand and the exportable surplus will most likely stay as the same.

Egg imports and exports – current and future outlook

During the remainder of this decade the outlook for the international **egg trade** is somewhat bleak, it will remain a buyer's market.



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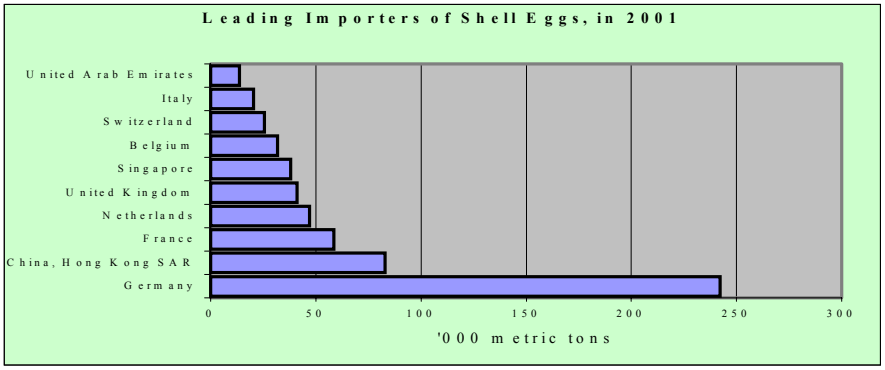
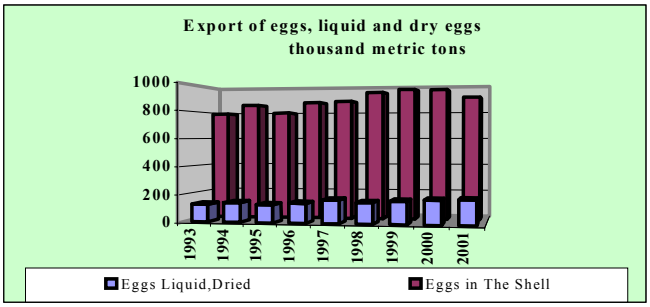


Including intra European trade, in 2001, the major importers of eggs were: Germany, Hong Kong, Netherlands, France, UK, Singapore, Belgium, Switzerland and Italy.
Some of these countries make up the largest egg exporters in the world: the Netherlands, Belgium, France, and Germany.

SHARE OF LEADING IMPORTERS (value) percent							
	1995	1996	1997	1998	1999	2000	2001
Canada	5	5	7	8	7	6	8
Hong Kong	14	13	13	12	13	12	13
France	14	14	13	13	13	14	16
Germany	50	50	51	50	47	45	47
Italy	7	8	7	7	6	14	7
Netherlands	10	10	9	10	14	9	9

In the last five years Germany has been the biggest importer of egg and egg products. From the total eggs imported, most went to the domestic market, the rest were re-exported. Other big importing countries in 2001 were Italy Hong Kong Netherlands and France.
The biggest exporter of eggs and egg products was the Netherlands, approximately



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37% of egg exports come from this country.
The egg external market is being concentrated into fewer hands, mainly the western European countries. Outside the EU, there are only two major buyers - Japan for egg products and Hong Kong for shell eggs; and the latter market will be taken by China.
The export of liquid and dry eggs represents only 15% of total egg exports.
European countries are both the biggest exporters and importers of liquid egg products. As the integration intensified, EU intra trade is harder to distinguish, especially the egg trade. According the FAO forecast results, EU, as a whole, will continue as the main supplier of egg to the world market, however it is expected to recede in year 2015 and then to continue increasing net export to world market by 2030. Comparing to USA's strong rising in net egg export to world market in the next 30 years, EU is losing the share of its egg market by 2030. Moreover, world egg market will start turning to be consumption driven by 2030 and the markets are still in developing countries, despite their expansion in production. Among the top net importing countries according to the forecast, Yemen and Hong Kong keep expanding, Canada and Japan are keeping the same and Switzerland's will drop. As for the net exporting countries, China, U.S.A and European Union countries will raise their export in the next 30 years.

NET TRADE

COUNTRY	1998/2000*	2015	2030
Eggs: thousand metric tons traded			
All developing	-32.7	-114.5	-221.7
Developed countries	75.7	158	221.7
Net Importing			
YEMEN	-10.2	-44.4	-113.7
CANADA	-31.2	-33.6	-34
JAPAN	-36.6	-50	-50
SWITZERLAND	-38.2	-32.3	-26.6
HONG KONG	-85.2	-101.4	-112.9
Net Exporting			
CHINA	57.1	59.6	62.5
USA	104.5	150	200
EU15	122.1	114.4	123.6

** Base for these calculations*

Some Points to Note:

1. Domestic margins are everywhere depressed.
2. The market is being concentrated into fewer hands.

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3. For Western Europe, there are regulatory problems, which could cost 5.7 billion Euros up to 2012 to remove cages.
4. There is also the view that operations may be moved out of high cost areas to other locations to save costs and avoid regulations.
5. The world trade in shell eggs is very small indeed, due to the nature of the product; many shell eggs are dumped in order to raise domestic prices. If 1% of eggs are exported, domestic prices could rise by as much as 5%.
6. Outside the EU there are only two major buyers: Japan for egg products and Hong Kong for shell eggs; the latter market will shortly be taken by China.

In summary, the outlook for the year 2015 is for a world egg market with considerable exportable surplus production, dominated by USA, Europe, China, followed with some distance by Thailand, Malaysia and maybe Turkey which will have export egg surplus. Sub Saharan Africa could be a net exporter of eggs depending on events in Nigeria but **Latin America, mainly due to expected strong demand in Peru and Central America, is forecast to be a net egg importer** . South Africa could potentially be an exporter of eggs in 15 to 30 years' time.

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EU ORGANIC POULTRY PRODUCTION AFTER THE EU REGULATION ON ORGANIC LIVESTOCK PRODUCTION

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Keywords: poultry, organic farming, EU Regulation

Abstract

Since 19 July 1999, EU Regulation EC 1804/99 sets the rules for organic livestock production in the European Union. The comparative study of the organic poultry standards in seven EU countries (United Kingdom, Germany, Denmark, Austria, Netherlands, Belgium, Sweden), followed by short stays in each of those countries, has enabled us to enlighten some major differences in the interpretation and implementation of the EU Regulation. This study has also brought to light some technical issues that are common to the different countries.

Résumé

Depuis le 19 juillet 1999, le règlement CE n°1804/99 (Règlement Européen pour les productions animales biologiques ou REPAB) définit les modalités des productions animales biologiques. L'étude des cahiers des charges en vigueur dans sept pays de l'Union Européenne (Royaume-Uni, Allemagne, Danemark, Autriche, Pays-Bas, Belgique, Suède) pour l'aviculture biologique, complétée par des missions dans chacun des pays, a permis de mettre en lumière certaines divergences majeures dans l'interprétation et l'application du REPAB. Cette étude a également permis de révéler certaines difficultés techniques rencontrées quels que soient les pays.

Introduction

On 19 July 1999, the European Ministers for Agriculture have adopted Regulation (EC) No 1804/1999 supplementing Regulation (EEC) No 2092/91 on organic production of agricultural products to include livestock production. This Regulation

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sets the rules for organic livestock production in the following fields: conversion, origin of the animals, feed, disease prevention and veterinary treatment, husbandry management practices, transport and identification of livestock products, livestock manure management, free range areas and livestock housing. This regulation stated that it should be possible for Member States to apply more restrictive rules for the livestock and the livestock products produced in their territory. This is the choice France made when they introduced a number of additional rules in their "Standards for organic livestock productions" (often called in France "REPAB-F").
France is the first EU country in the field of organic poultry production with, in 2000, 1.4 million laying hens and 5.7 million table chickens. But since the REPAB-F has come into force, the development of French organic poultry production has stopped. Also, members of the French poultry industry fear that the additional constraints of REPAB-F might reduce the competitiveness of their organic products on the EU market. That's why the French industry have asked for a study that would analyse the situation of the organic poultry sector in seven EU countries : Austria, Belgium, Denmark, Germany, the Netherlands, the United Kingdom and Sweden. The purpose of the study was to describe this sector in each country and to estimate its competitiveness and development prospects.
In this article, the aim is to enlighten some major differences in the interpretation of the EU Regulation concerning poultry production through a few examples and to describe some common problems encountered throughout the EU by poultry producers.

Material and Methods

The study consisted of a comparative study of the organic poultry standards in seven EU countries followed by short stays in those countries to analyse the implementation of the standards. The study was conducted between March and September 2002.

Results and discussion

Diverging interpretations of the EU Regulation

Relationship between livestock production and land and conventional animal husbandry on organic farms

EU Regulation states that "organic production of livestock must in principle provide for a close relationship between such production and the land […] and *the feeding of livestock with organic-farming crop products produced on the holding itself*".

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Complying with the principle stated here, French REPAB-F requires that at least 40% of poultry rations should be produced on the holding. This requirement poses a problem for many farmers. Some of them don't have enough land to produce 40% of the feed. Others have enough land but they don't want to convert their land to organic agriculture (too much work, too requiring, *etc*.). In Austria, Germany, and Sweden 50% of the feed have to be produced on the holding. In Germany, it is possible to cooperate with another farm to produce the 50% feed (feed-manure exchange).
Moreover, the REPAB-F states that it is not allowed to have on the same farm animal reared conventionally and organically ("parallel production")[1]. This is also a limiting factor for the development of organic poultry production since not all organic poultry farmers are ready to convert their other livestock. The ban on parallel production is also required in some others EU countries: Austria, Denmark and Germany.
In Belgium, in the Netherlands and in the UK, there are no requirements of autoproduction of feed nor whole conversion of livestock.
The question of inside stocking densities for laying hens
In the EU Regulation, it is stated that the maximum stocking density for laying hens is 6 hens per m² "net area available to the birds". This ambiguous phrase lives space for various housing systems and calculation methods.
In deep litter systems, the stocking density can be calculated taking into account the ground area of the poultry house or the "usable area"[2]. According to the country or the control body, it is one or the other definition that is used. In aviary systems, the area considered is the "usable area" or the "net area available to the birds". The area of the several tiers is counted in. Thus it is possible in some countries to have less than 6 birds per m² usable area in an aviary, in fact equal to 10, 12 or even 15 birds per m² ground area. Aviary systems exist in Germany, Belgium and the Netherlands. Aviaries can result in reduction of heat costs (thanks to the increased stocking density), but also of feed costs by improving the feed conversion. They also reduce labour costs and building surface. However, aviaries don't reduce the investment cost per hen (building costs are reduced but equipment costs rise).
The use of a winter garden is frequent for laying hens and tends to generalise. In Germany and Austria, winter gardens are also used for table chickens. It is a space that offers protection from rain and wind, covered with litter material and lit with daylight. It is between the building and the outdoor area. Most of the time, the winter garden area is counted in the area of the poultry house if it is accessible to the birds all day. It is not the case in France: until now, winter gardens were not counted in the calculation of the stocking density. In the UK, the winter garden sometimes is one third to one half of the total area and it is in some cases the only space covered with litter (the

[1] The presence of non-organic animals is tolerated until 24 August 2008.
[2] According to the Directive 1999/74/EC on the welfare of laying hens, usable area is defined as a surface of minimum width of 30 cm, a maximum slope of 14% and minimum height of 45cm; nest area should not be included.

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inside of the building is completely slatted)[3]. When the size of the winter garden is equal to the size of the building, this means that the stocking density in the building is double if one considers the French calculation method.

Number of birds per building

The REPAB-F imposes a maximum of 3 000 laying hens and 4 800 chickens per building. In the original version of the EU Regulation in English, it is not the word "building" but the word « poultry house » that is used. Using the second concept ("poultry house"), some countries allow buildings containing several poultry houses. Thus, in a number of countries, the maximum size of 3 000 hens and 4 800 chickens is not interpreted as a number of animals per building but as a flock size, and several flocks can be in the same building. In Germany and Austria, the interpretation is the same as in France. However, some German buildings under derogation[4] will be used until 2011. What's more, the permanent possibility to cumulate flocks in one building gives an advantage to UK, Danish and Dutch producers.

The question of slaughter age for organic chickens

The minimum slaughter age imposed by the EU Regulation is 81 days, except if slow-growing strains are used. In this case, it is possible to slaughter before 81 days. French producers do not understand the logic behind this sentence. Moreover there is no definition of what a "slow-growing strain" is. In France the average age at slaughter is close to 90 days, due to the fact that the French regulation imposed a minimum age at slaughter of 91 days before the EU Regulation came into force. In Germany, Austria, and in the UK, birds are slaughtered before 81 days. Since there is no definition, of "slow-growing strains", producers in those countries consider that the strain they use enter this category. Arguing that they are using strains growing slowlier than "industrial" strains, producers can slaughter before 70 days[5] if they use certified organic chicks. This is already the case in Germany and will soon be the case in Austria. One can foretell that the same kind of approach will be chosen in the UK where some farmers already have too heavy chickens at 70 days. Despite the cost of an organic chick, the possibility to slaughter earlier will give an important competitive advantage.

Common problems concerning laying hens rations

The lack of good quality organic protein sources is felt everywhere. Therefore protein and essential amino acids needs of the laying hens are difficult to cover with rations complying with the restrictions imposed buy the EU Regulation, and especially because of the ban on synthetic amino acids. This can foster pecking and cannibalism,

[3] EU Regulation states that at least one third of the building shall be covered with a litter material such as straw, wood shavings, sand or turf.

[4] EU Regulation authorises derogations from the requirements on housing and free range areas for a transitional period expiring on 31 December 2010, for holding with existing buildings, constructed before 24 August 1999.

[5] 70 days = 10 weeks conversion for conventional chicks to become organic birds

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increase mortality (compared to conventional free-range) and contribute to decrease laying performances. From one country to another, the position on this topic differs.
In France, producers would like the synthetic amino acids to be reintroduced. It is also the opinion of Belgian feed compounders who, through their union Probila-Unitrab, have asked their Ministry to allow synthetic amino acids. In the UK, authorities tolerate the use of synthetic amino acids and the use of synthetic amino acids is quite widespread. Dutch Ministry for Agriculture has also declared they were in favour of a temporary reintroduction of synthetic amino acids in organic layer rations. The Austrian Ministry in charge of the EU Regulation supports the ban on synthetic amino acids while Austrian farmers and feed compounders are more divided. In Germany, people think that the ban on synthetic amino acids could be a problem in the future ; as a matter of fact, conventional maize gluten, potato protein and brewer's grains currently make it possible to cover the protein needs of the animals. But it will be more difficult (and more expensive) after 2005, when conventional raw materials are not allowed in organic rations.
The difficulty to satisfy birds needs in proteins and essential amino acids is increased by the ban on meat and bone meal and, in many cases, the impossibility to use fish meal in poultry rations (problem that does not only concern organic production). The use of fish meal in organic rations is explicitly forbidden in Belgium, in Germany[6] and in Austria. In France, in the Netherlands and in the UK, the legislation makes it difficult for feed compounders to include fish meal in the rations. On the other hand, in Denmark, fish meal is in general used.
The problem of organic pullet and chick production
EU Regulation states that from the 1 January 2004 pullets will have to be reared organically from day-old chick, and chicks used for organic chicken production must be organic. However the EU Regulation does not set standards for pullet rearing and does not define what an organic chick is.
A few countries have already started a production of organic pullets: Austria, Denmark and Germany. In Belgium, pullets are reared organically from week 6. At the time of the study UK and Dutch organisations were working on standards for this production. Up to now, nothing has been done in France on this subject. The German are the more advanced on this point since 100% of the production should be using organic pullets by 2003. These pullets eat organic feed from day 1 and have access to a winter garden. Access to an outdoor range is not compulsory. Already, differences can be seen between standards for pullet rearing (or draft standards) in different countries.
Production of organic chicks in the EU is currently very limited. The production has started in Austria, in the UK (although standards are not written yet) and in Germany. In all countries without exception, producers are worried about the standards to come. The majority consider that the fact of giving access to outdoors to parent flocks would

[6] (except for starting turkeys in Naturland standards)

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pose very serious sanitary problems (contamination with Salmonella, Mycoplasma, *etc*.) with considerable economic consequences.

Conclusions

This study has brought into light the diversity of interpretations and implementations that can be made of the same text, EU Regulation No 1804/99, and the difficulty to harmonise a production at EU level.
When REPAB-F was written, France wished to promote a certain model of organic agriculture. This study shows that the French interpretation of the EU Regulation is on some points penalising for the French production in terms of cost of production. Nevertheless, the development of a local production in many countries traditionally supplied by France also explains the loss of many export markets.

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EUROPEAN HEN WELFARE AND COMPETITIVENESS OF EGG PRODUCTION

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Keywords: eggs, hens, welfare, production-cost, competitiveness

Abstract

The conditions under which laying hens are kept are still a major animal welfare concern. The number of animals involved is very high and it is one of the most intensive forms of animal production. Directive 1999/74/EC laying down minimum standards for the protection of laying hens allows three different categories of farming systems: alternative systems, enriched and unenriched cages. The dominating system for the production of eggs in the EU used to be the so-called “unenriched cage”, which is actually going to be banned by 1st January 2012. The use of cages improved by environmental enrichments (so-called “enriched cage” system) is still marginal. In some countries (Sweden) traditional cages are banned earlier (2004) and Germany is going to ban traditional cages from 2007 and enriched from 2012. Alternative systems are used in the form of free range and barn systems. For the marketing of “free range eggs” Commission Regulation 1274/91 sets out additional conditions for the production system.

Résumé

Les conditions dans lesquelles les poules pondeuses sont élevées sont toujours une préoccupation importante de bien-être des animaux. Le nombre d'animaux impliqués est très élevé et il est un des modes le plus poussé de production animale. La directive 1999/74/CE fixant des normes minimales pour la protection des poules pondeuses permet trois catégories différentes de systèmes
d'élevage: les systèmes alternatifs, les cages et les cages aménagées. Le système prédominant pour la production des œufs dans l'UE est la cage traditionnelle, qui sera interdite à partir du 1er janvier 2012. L'utilisation des cages améliorées par les enrichissements environnementaux (dénommés le système des cages aménagées) est encore marginale. Dans certains pays (Suède) les cages traditionnelles vont être

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interdites plus tôt (2004) et l'Allemagne va interdire les cages traditionnelles à partir de 2007 et les cages améliorées à partir de 2012. Les systèmes alternatifs utilisés pour élever les poules sont les systèmes en plein air et au sol. Pour la commercialisation des œufs produits en plein air, le Règlement de la Commission 1274/91 expose des conditions supplémentaires pour le système de production.

Background

The condition under which laying hens are kept is still a major animal welfare concern. The number of animals involved is very high and it is one of the most intensive forms of animal production. Directive 1999/74/EC laying down minimum standards for the protection of laying hens allows three different categories of farming systems: alternative systems, enriched and unenriched cages.
The provisions of the Directive are being progressively implemented since 2002. They introduce technical changes in the current systems (unenriched cages and alternative systems). Since there is only limited practical experience with the production in enriched cages (Sweden) and since modifications to the current systems have been adopted, the knowledge on the economic implications of the different farming systems needs to be updated. In order to have an overview of the current situation, the study of the balance between welfare, hygiene, economy and environmental implications and its impact on the cost of production and on the competitiveness of EU production with third countries is considered a priority.

Analysis of the current situation

Currently, the predominating system for the production of eggs in the EU is still the so-called **"**unenriched cage**"**. Moreover, starting from 1st January 2003, all cages should be enlarged from 450 cm² to a minimum of 550 cm² and this minimum space should be available per laying hen at all the time. The minimum standard should be kept in place up to year 2012 when all un-enriched cages should be banned. The EU egg producers will be probably adjusting to this ban gradually. The use of cages improved by environmental enrichments (so-called "enriched cage" system) is still marginal. These improved cages could be an alternative after the ban of traditional cages in 2012. Each lying hen should be kept individually in enriched cages, with 750 cm² of space available at all the time and some facilities as perch, nest and litter, to allow the bird to perform with its natural behaviour. As from first January 2003, no other than enriched cages may be newly build.

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More alternative systems are the free range and the barn systems. For the marketing of "free range eggs" Commission Regulation 1274/91 sets out additional conditions for the production system.
The increase of the production costs comes from different factors. The criteria to consider are several, as lower number of hens in the cage, higher cost of enlarged and enriched cage in respect to traditional cage, the greater feed requirement per hen when the bird can move and perform more. Switching to barn and free range systems, important factors involved in the increase of production cost exist. They are: -the freedom of movement and the possibility to access to outdoor areas that make the feed conversion higher reported to the production performances; -the reduction of control over the feed intake; -the greater mortality; -the increment of veterinary and medicine bills; -the higher labour costs. According to a recent study, carried out by the "Research Institute for Animal Husbandry" of Wageningen (NL) executed at 17 poultry farms and included 410,000 free-range layers, the mortality rate was concluded to be a high 14.3% due to mainly E-coli infection. The number of eggs laid outside the nest varied form 0.4 to 5.6% with an average of 2%. A similar study also revealed that "organic" layers have more endo-parasites than battery hens but suffer less form blood lice. It is easy to understand that all these factors are going to have quite a sensible impact on the production costs.
This cost increment will reflect on the purchaser, and the question is how much more the consumer is ready to pay for the welfare of the hen. It is open whether producers of different size (small-medium-large) will be able to complete the necessary investments in time to improve the welfare of their hens. In all cases, a financial aid could be helpful to maintain a balance between farms of different size.
In fact, there are more implications that should be taken into account. The economic impact of the welfare standards will affect even the competitiveness of the entire EU industry. In most third countries in the world, the animal welfare standards have not such a high standard as in EU. On one hand, the consumer is looking to have an egg produced under certain criteria and, on the other hand, when is going to purchase a product, it could be mostly common that he/she will go to buy the cheaper one. It is clear that the consumer is ready to pay something more, but not a double price for example, especially in this particular period of recession. It appears like a gap exists between what the consumer requires and what is ready to pay. This attitude could cause an increment on the import flow of non-EU eggs, third countries being, in many cases, more competitive. For example, a third country supplier as the US will be able to offer at lower cost even eggs produced by hens kept in enriched cages.[1] Moreover, two determinant factors to evaluate the competitiveness between EU and third countries are the level of import duties and the different currency exchange rates. The

[1] Eurogroup for Animal Welfare, Brussels 2002.

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World Trade Organisation' policy is clearly in favor to liberalise more the trade of agriculture products and the Euro can be considered quite a strong currency, especially when compared with the currencies in developing countries. In this scenario, the shell egg sector will be, to some extent, protected from a great increase of import, because of the fragile nature and the need for freshness of table eggs and the high cost for transporting the liquid part of the egg. For egg products, the situation is different and the sector is not as protected as the previous one. The risk of loosing competitiveness, due to the increment of cost to reach the welfare standards and to the other factors mentioned above, as the lower transport cost, is much higher. Moreover, consideration should be given to the fact that table eggs have always had a big part of the market but that egg products have been increasingly taken more space in the market within EU, as a convenience food. This change was due to the social tendency to have more single person households, more women working, more fast foods and, if this tendency is going to continue, it will have major consequences on the competitiveness of the EU and on the import flow.

Costs

Some figures available show that in Europe, at the moment, a high percentage of eggs are still produced in cages. On the other hand, some Member States have already switched to alternative methods, as Denmark, Sweden, the Netherlands and UK. The reluctance of some countries to pass to the alternative methods could be based on the easy way to manage a battery-cage farm, the safer animal health situation and the lower production cost.
The data available at the moment, coming from three European sources, show the following percentage of differences in production costs:

- First source: total equipment + poultry house in Euro per hen place. The cost of a traditional cage of 450 cm² is € 17,5 and the related production cost of one table egg is 0.044 €/egg. An enlarged cage of 550 cm² will increase the cost of production of +8%, giving 0.048 €/egg. The enriched cage costs 27,9€ (+58.5%) giving 0.05€/egg (+13.6%). The barn system will increase the cost per hen place by +48.8% and production cost will rise to 0.052 €/egg (+ 18.6%).[2]
- Second source: Traditional cage of 450 cm², production cost per/egg=0.048 €. Barn system, production cost per/egg=0.060€ (+25%). Free range, production cost per/egg=0.077 € (+60%)[3]

[2] 2001: P. van Horne-Wageningen University.
[3] Eurogroup for animal welfare.

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- Third source: a 1995 study shows the following production costs: conventional cages (450 cm²) = 0.062 €/egg. Barn system = 0.079 €/egg (+27%). Free range system = 0.093 €/egg (+ 50%). An egg produced under organic rules would cost 0.14 €, which increase the price by 125%. The high production cost of organic eggs does not come from high animal welfare standard but originates for a very large extent from the high prices for feed and fodder, that are three times the price than in conventional production.[4]

It is now interesting to compare those figures with the US production costs. Using the traditional cage, a US farmer produces a table egg at the cost of 0.0355€, which is 80.5% of the production cost/egg for a producer in the EU. The difference is based on the feed cost, pullet cost, housing etc.

Outlook

In order to have an overview of the current situation, EU studies of the balance between welfare, hygiene, economy and environmental implications and its impact on the cost of production and on the competitiveness of EU production with third countries have been undertaken. They are needed for preparing the review 2005 on welfare standards, taking also into account the World Trade Organisation results and the study scientist welfare.

[4] 1995: Welsh Institute of Rural Studies.

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CHANGES IN DEMAND FOR EGGS AND EGG PRODUCTS AND INFLUENCE OF CHANGES IN WELFARE REGULATIONS

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Keywords: eggs market sectorisation, consumer expectations, animal welfare

Abstract

The strategies being developed by members of the egg industry are strongly influenced by changes in regulations concerning welfare of laying flocks and egg labelling, and by new consumer expectations. Freshness and food safety remain the main criteria for purchase, and increasing consumer awareness of farming methods is likely to affect these criteria. For the domestic consumer the degree of willingness to pay more for welfare gives hope for the development of systems providing guarantees on farming methods. On the other hand, the demand expressed by the catering, restaurant and food industries for systems guaranteeing animal welfare is very low.

Résumé

Les stratégies développées par les acteurs de la filière œufs sont fortement conditionnées par les évolutions réglementaires, concernant le bien être des pondeuses et l'étiquetage des œufs, et par les nouvelles attentes des consommateurs. Fraîcheur et sécurité alimentaire demeurent les principaux critères d'achat. La sensibilisation croissante des consommateurs citoyens aux modes d'élevage est susceptible de faire évoluer ces critères. Sur le marché des ménages, la mesure du consentement à payer pour plus de bien être laisse espérer un développement des systèmes apportant des garanties sur le mode d'élevage. Sur les marchés de la RHD et des IAA, la demande exprimée pour des systèmes garants du bien être animal est par contre très faible.

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Introduction: Background and Aims

In the context of the implementation of the European Welfare Directive (Directive 1999/74/CE), which seeks in due course both to do away with conventional cage farming and to encourage the development of alternative systems, it seems essential to understand the precise expectations of the various egg users. The factors in the evolution of demand are based on the underlying expectations of the end consumers, on the constraints affecting supply by generating extra costs and on the willingness of the consumer to pay more for animal welfare. It is a question therefore of assessing both the impact of the farming method and the differentials in production costs on consumer behaviour and those of industrial users of eggs and egg products. We shall therefore seek to define the current image of farming systems, the expectations of the various users, including food safety and egg qualities related to production methods, and the price increases related to these different levels of quality.

- **Analysis of quantitative changes in demand for eggs and egg products and the current sectorisation of the market**

With 15.2 million eggs produced for consumption in 2002, France supplies about 18% of the eggs produced in the European Community (84 billion eggs). The increase in production in the past twenty years is related to development of the egg products' sector, with increasing demand in the home and export markets. The production of egg products reached 231,000 tonnes liquid equivalent in 2001 (270,000 tonnes in-shell equivalent, i.e. 29% of production), an increase of 33% compared to 1998. A second line of differentiation occurred at the end of the 1980's to meet the new demand for 'free range' and more recently organic eggs from French consumers. Such alternative farming methods represent about 12% of the livestock and about 10% of French production.

The Frenchman remains one of the greatest consumers of eggs in the European Union (250 eggs consumed per person in 2002, vs. 213 in Europe). About a quarter of egg consumption comprises egg products used by the food industry, by food craftsmen and by the catering and restaurant industries. Direct consumption continues to represent about 10% of overall French consumption, whereas the purchase of in-shell eggs represents about a quarter of total consumption and domestic purchasing about 40%.

Purchasing of in-shell eggs by domestic consumers is decreasing at a rate of 1 – 2% per year, whereas the value of sales is increasing. These divergent trends illustrate two main axes of increasing sectorisation of the egg market in supermarkets: guarantee of freshness (dated and extra fresh eggs) and farming methods (mainly free-range and organic). Another aspect of sectorisation of the market is the increasing importance of retailer own brands, which currently represent 40% of supermarket egg sales.

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According to SECODIP, in-shell eggs represent a significant proportion of turnover (49%) within the catering and restaurant market (922 million francs turnover in 2001). This is followed by cooked eggs (35% of turnover), which includes shelled hard boiled eggs, precooked omelettes, poached and scrambled eggs, and sliced hard boiled eggs. The use of liquid eggs is expanding, but they are only in third place, with 15% of turnover.

The future of egg consumption in France is more qualitative than quantitative: development of egg products (certain sectors such as food artisans and catering and restaurant trades, still low users, should be changing gradually to egg products), and the greater of consumption of free-range and organic eggs.

- **Impact of layer farming methods on consumer demand**

Although several studies on the consumer's image of eggs (Agro Service Etude and Itavi, 1996, Imaj 1996) have confirmed that the egg, as a basic, multipurpose foodstuff, symbol of life and reference food, has an excellent public image (healthy, natural food), very few studies are available on consumers' perceptions of farming methods or on the impact of these factors on purchasing behaviour.

With the financial support of French Ministry of Agriculture (DGAL) and French Interprofessional Council (CNPO), ITAVI therefore performed a study in 1999/2000 to attempt to answer these questions. The aims of the studies were initially to evaluate the impact of understanding of farming methods on the perception of available and future eggs by placing consumers in simulated purchasing situations. Four meetings of consumer groups were organised in November/December 1999 with the cooperation of a private market research group (GEM). Purchasing behaviour was tested twice in each group: the first time at the beginning of the session, with a simulated current shelf line, and the second at the end of the session (before or after provision of information on the "new farming methods") with a line corresponding to a "2012 product" (without French eggs produced in cages, with enriched cages, aviaries, etc). This was followed by an opinion poll by the BVA (a market research organisation) in a representative sample of approximately 1,000 people.

- **Purchasing criteria: current situation**

This study provided an evaluation of consumers' purchasing criteria and revealed a discrepancy between objective purchasing criteria (purchasing behaviour) and the attitudes expressed on these criteria by consumers. This was particularly true for criteria related to farming methods, frequently given as purchasing criteria, but which did not feature in actual purchasing behaviour.

The main objective purchasing criteria, analysed on the basis of purchasing behaviours and demonstrated with the simulated shelf lines, emphasized the importance of the ultimate use of the eggs:

- Packaging and size
- Freshness, although this was difficult to define explicitly and objectively

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- Confidence, the main features of which were personal identification of the vendor (market, farm) and Brand name in supermarkets.
- Price

These results were generally confirmed by the quantitative study (attitudes).

Ranking of purchasing criteria (n=959)

I am going to read you various criteria of egg purchasing. For each one please say whether you consider it to be very important, quite important, not very important, or not at all important if you have/had to buy eggs.

	"Very important" and "quite important" replies	*%*
*	Freshness of eggs	98
*	Guarantee of healthy eggs	95
*	Farming method for laying hens	71
*	Type of layer feed	71
*	Welfare of layers	70
*	Quality mark on packaging	61
*	Price	59
*	Brand name	25

Freshness and guarantee of healthy eggs were the main criteria for 95% of those questioned. These were followed by three criteria (farming method, feed and chicken welfare), which together made up about 70% of replies.

The qualitative and quantitative approaches were not in agreement on the importance of a brand name, which was considered to be very important for the shelf line and hardly featured in replies to the opinion poll. This result also illustrates the discrepancy between attitudes and behaviour in the simulated situation in the qualitative study. The latter provided a basis for dividing consumers into three types.

- Organic influence.

Such consumers are highly informed and are very aware of free-range rearing. They buy organic eggs in supermarkets or comparable eggs sold as farm eggs in markets. Expression of their purchasing criteria and their off-the-shelf behaviour are consistent. They are the only consumers to give farming method as a determining criterion.

- Battery farming-sensitive consumers

These consumers, who form the majority, are strongly opposed to battery farming. However, the farming method does not feature in their actual purchasing criteria, and there is considerable contradiction between their attitudes and their purchasing behaviour. This contradiction can be interpreted as resulting from two currently distant spheres between which the consumer alternates, i.e. that of world citizen (indignation with intensive farming methods, which are considered as against nature) and that of

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consumer for whom the importance of the final use (freshness, size, brand name) determines purchasing behaviour.

- Non-aware consumers

Such consumers, who are in the minority, are interested only in egg quality, purpose of use and price.

- **Image of the different farming methods**

A marked dichotomy has appeared within the first two groups, with a clear conflict between two images: battery (industrial world, prison-like, "inhuman", not respectful of animal welfare, and suspect in terms of quality of the eggs produced) and farm eggs (idealistic reference image). The other farming methods (free-range, hen-houses, etc) give rise to more questions and are subject to obvious lack of knowledge: the nature of the information provided can easily change consumers' opinions. Even organic farming, which enjoys an image of rigour and morality, raises questions: how is it possible to reconcile a quasi 'cottage industry' with constant presence in supermarkets? It is difficult to choose between aviary/ deep litter/ enriched cage systems; divisions are more clearly made between access to outdoor facilities or not. The organisation of "hen houses" is of little interest to the consumer but, on the other hand, concepts such as group size and density can be discriminating factors.

These different features were confirmed by the quantitative study.

The consumer wants safe eggs, and the citizen believes that a factory farming system is not able to produce them. Any system which reinforces health safety guarantees (labels of origin, brand names, etc) is of course perceived as favourable by the consumer. There is at the same time a confusion in the minds of consumers who liken health guarantees to traditional farming. It is thus possible to anticipate that any crisis or information which challenges this oversimplification and the indissoluble relationship between health safety and alternative farming methods will result in rejection of the system in question, and probably also the whole product. The study performed by TMP in October 2000 showed that systematically putting "eggs from battery-farmed birds" on packaging would result in consumers switching to eggs produced by other farming methods (72% of consumers) or to a reduction in consumption (24%).

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Consumer image of different farming systems (n=959)

I am going to read you some sentences, which describe each farming system. Please would you tell me which sentence corresponds completely, more or less, not really or not at all to what you think about battery farming of hens?

'Completely' and 'more or less' replies.	*%*
Battery farming	
It is an inhuman prison for birds	87
Battery farming provides cheap eggs	83
The system is incompatible with reasonable living conditions for hens	83
It is an economically rational system	61
Battery farming produces safe eggs	27
Free-range farming	
It is a farming system which produces safe eggs	85
It is a traditional farming system in which birds are free/run around freely	84
It is factory farming with a label of origin which sells better	68
It is factory farming in which birds can go outside at certain times of the day	65
Organic farming	
It is a farming system which produces safe eggs	76
It is a farming system in which birds are fed organic feed	76
It is a traditional farming system in which birds are free to roam	68
It is a production method which is good for chicken welfare	65
It is factory farming with a label of origin, which makes selling easier	60

- **Anticipated behaviour with "2012" products**

When faced with the anticipated future lines, there was consistent behaviour with regard to extreme categories (organic and cheapest products), the most marked being consumers looking for additional guarantees in terms of freshness and food safety. After receiving information, the latter are likely to change to free-range, after having superimposed the idea of battery production on the eggs they bought before. The group sessions as a whole tended to show a probable shift towards consumption of free-range and organically produced eggs, encouraged by media campaigns or simply by information about farming methods which tend to bring the "consumer" and "world citizen" closer together. Only a minority of consumers show willingness to pay almost nothing for greater welfare or naturalness (the two being strongly related). Indeed, the low unit price per egg indicates that it will be easier to obtain acceptance of an increase related to changes in farming methods. However, sectorisation and clearly differentiated supply appears to be advisable for most suppliers.

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Willingness to pay for improvements in chicken living conditions

A standard extra-fresh egg from battery hens costs about 1 French Franc at the moment. What would you be prepared to pay for an extra-fresh egg from laying hens in improved living conditions?

	%
1 F F	18
1.01 - 1.49 FF	17
1.50 FF	22
1.51 – 1.99 FF	3
2.00 FF	18
More than 2 FF	9
No comment	13

It is perhaps necessary to compare these results with the figures for the groups identified during the qualitative study. In fact, the two studies were relatively consistent in demonstrating three groups: the non-aware or poor, who would not agree to pay any more for welfare (18%), the organic supporters who are partly included with those who say they are prepared to pay twice as much (27%) and those who are aware, i.e. the majority, and say they would be prepared to pay 0 – 50 centimes more for an egg (40%). These figures are not far from the existing proportion of the market for the cheapest eggs (12-15%) and alternatively-produced eggs (15%), which indicates that a certain amount of change is possible in this sector if the 27% of consumers stating agreement to pay 2F or more are taken into account. The qualitative study also introduced a more subtle concept, i.e. that of flexible purchasing. Indeed, consumers do not have a univocal view of eggs. It is a food ingredient among many others on the one hand and a symbol of life on the other, and the consequence is that the type of purchase will also be depend on the intended method of consumption of the product.

Willingness to pay must generally be viewed with caution because, as shown in a study by Burrell and Vrieze (2001), there is no link between awareness of welfare issues and willingness to pay, or of willingness to pay and the purchase of alternative types of eggs. There are other sociological variables that explain this type of purchase. Similarly, the authors suggest that there is not a high correlation between the importance given to the farming method and willingness to pay.

- **The industry's view of changes in the ultimate demand and the market**

After evaluating consumers' expectations concerning egg quality by investigating attitudes and purchasing behaviours, it is necessary to compare the results obtained with the view of the industry's members of the market and the future. Nearly twenty interviews took place between February and December 2000 to define the current sectorisation of the egg and egg product markets and the advantages and disadvantages

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of the various sectors, and to make a forecast for 2012 on the basis of the opinions and strategies already announced and the time scale of the various regulations.

- **View of changes in demand for in-shell eggs for domestic use**

According to the suppliers interviewed, consumer expectations focus mainly on food safety, freshness and price. According to the distributors, the effect of price on consumer behaviour is different according to the types of customer and the policy of quality differentiation they apply. A small number of retailers anticipate that some of their customers will be poorer and the price criterion will become more important. The lowest priced eggs (sold for 35-60 centimes) represented 12% of sales in 2000 and, according to this hypothesis, it could increase to 20% by 2012. The increasing proportion of the market held by Hard Discounters in egg market tends to confirm this.
The members of the industry interviewed, including distributors, supported two ideas revealed by the consumer studies. The expectations of consumers vary according to the group to which they belong (types revealed by consumer studies). On the other hand the concept of "free-range" is strongly associated with that of freshness. These consumer concerns are generally reflected by the requirements of distributors, particularly expressed in the specifications of products bearing the distributors own labels. Layer feed and food safety are among the main elements listed in the specifications. The long-established practice of dating eggs with the day of laying represents 20% of the overall market and is evidence of the importance given to freshness. Other specifications mainly focus on traceability at packing stations.
The representatives of the distribution industry interviewed agreed almost unanimously on the following hierarchy of current French consumers' expectations and on future sectorisation of the market:

1 Freshness, already well established as a requirement and remaining predominant, is evidenced by replacing 'extra-fresh eggs' by dating of eggs.
2 Traceability of animal feed (guaranteeing the absence of animal meal and GMO) and finished products, which can substantially affect brand supply policy by encouraging the setting up of, dedicated farms and packing stations.
3 Appropriate packaging, particularly for city populations where household size may be one or two people.
4 Animal welfare, the importance of which is being increasingly recognised by certain brands.

Egg producers and distributors thus agree in recognising that in France animal welfare or farming method comes well behind food safety, freshness and feed for layers among consumers' concerns, even though consumers are becoming more and more aware.
For each sector (existing, emerging or possible in the long term) we sought to identify the advantages and disadvantages (handicaps and development factors) as they are currently experienced by suppliers and distributors. We summarise here the main

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conclusions providing projections for sectorisation of the egg industry in 2012. The distribution of the market into different sectors will be changed slightly: 'alternative' eggs, which in 2000 represented barely 15% of egg market, may reach more than 50% in parts of the market in 2012 (including barn eggs). This strong development, expressed, as a percentage of total volume marketed, will probably be less obvious if volume is considered per sector, in view of the considerable reduction in total sales to domestic households anticipated and probably enhanced by future regulations.

The expansion of sales of 'alternative' eggs will be accompanied by expansion of a new "top of the range" sector: expansion of sales of organic eggs will probably be limited by the constraints imposed by the new specifications and there will be strong competition at the top of the range due to the impetus of Label Rouge eggs which, according to the industry, may reach 15% of the market. This sector is currently benefiting from various development factors, e.g. probable conversion of certain Organic laying units to Label Rouge, the low additional cost of production compared to eggs from ''free-range' birds, and the enhanced value of the eggs. In competition with Label Rouge eggs, the marked for eggs from 'free-range' birds, the image of which seems less clear than that of organic or Label Rouge eggs, will probably be subject to more moderate development, and may reach 20% of the market.

Another currently emerging sector is that of eggs from deep litter raised birds. Various factors will influence the eventual outcome of this sector, among which is the competition of aviary system compared to deep litter, both from the point of view of cost and of image. The price competition of aviaries will depend mainly on the technical expertise of such farming in the years to come. The advantages of deep litter compared to aviary in terms of image are at the moment relatively small. However, such methods are likely to benefit from an image of less intensive farming, in view of the density differential between the two systems. We estimate that barn eggs should represent 20% of the market in 2012.

In view of the different hypotheses, eggs produced by the most intensive systems will still represent 45 – 50% of in-shell egg sales to domestic households. According to certain members of the industry, 10 – 15% of the market may be provided for by imports from other countries in the European Union, which are more effective in certain farming methods, or from central and eastern European countries, which will still not be required to respect Community restrictions. The remainder (30 – 35%) will be produced in enriched cages or aviary system, according to the price competitiveness of the two systems.

- **Changes in the demands of the canteen, restaurant and food industries**

According to the opinions of suppliers to the canteen, restaurant and food industries, where we have already seen that eggs are mainly supplied in their shells, food safety and price remain the main criteria.

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It does not seem at the moment that a demand has been expressed concerning laying hen welfare, apart from certain British clients and a demand for organic eggs from some school canteens. Some head offices for the canteen and restaurant industry may also eventually include welfare criteria in their specifications. Although it is difficult to measure, willingness to pay for greater animal welfare on the part of this industry is likely to be low, and certainly lower than that of the end consumers.

The main outlets for manufacturers of egg products are the food industry (liquid and dried eggs) and canteens, which use both liquid eggs and cooked egg products (hard-boiled, poached and scrambled eggs, and precooked omelettes, etc).

The quality criteria for eggs destined for the egg products' industry remain (in order of importance):

1 Food safety
2 Functional quality, mainly dry matter content
3 Traceability
4 Feed for laying hens (traceable feed, guaranteed without GMO)

Price remains, of course, an essential criterion, particularly in periods of higher prices, which sometimes force the food industry to import from the European Union or other countries. Animal welfare is rarely a criterion advanced by clients of egg product manufacturers, except in very specific circumstances such as organic food products.

According to the representatives of the food industry interviewed, 'alternative' egg products represented in 2000 less than 1,000 tonnes liquid equivalent from the total production of 220,000 tonnes, i.e. less than 0.5% of national production. The effects of the recent changes in the French specifications for organic poultry products and the resulting constraints have been reduction in supply and loss of competitiveness to competitors with less constraining specifications. This differential in competitiveness penalises exportation by French manufacturers of organic egg products. Finally, it may be in the long term that the French food industry imports a proportion of the organic egg products that it needs and that French suppliers cannot meet.

The qualitative changes expected in the demands from the canteen, restaurant and food industries are fairly low and leave little room to take into account farming methods and animal welfare. Microbiological and functional qualities, traceability and price competitiveness will probably remain the key factors to success with these outlets in future years.

Conclusion

Consumer studies have reconfirmed the pre-eminence of demands for food safety and freshness above all other expectations involving eggs. They have moreover revealed increasing awareness of the majority of consumers of farming systems, an overall

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concept which includes farming conditions, animal welfare, feed, animal health, size of unit and even respect for the environment. Farming methods can also be placed on a "freedom axis" which can then be superimposed on a "food safety" axis, setting "farm" against "battery", with free-range farming and one end (fresh, healthy eggs) and battery farming at the other (factory farming in which food safety is suspect).
The contradiction that exists between the high levels of consumer awareness of farming systems and purchasing behaviours, influenced by intended use of the product, can be interpreted as the coexistence of two distinct spheres for the moment, one as "world citizen" and the other as "consumer". However, the equilibrium is precarious, and discussion regarding layers farming and generalised information on type of farming method on packaging are likely to move the two spheres closer together, with a return to the importance of "farming method" in the group of determining criteria. As shown by the TMO study, the consequence will probably be an overall decrease in consumption.
Willingness to pay appears to be barely a relevant indicator because it is poorly correlated with awareness of welfare or actual purchasing behaviour. Estimates resulting from the study give reason to hope, however, for expansion of alternative 'top of the range' sectors, since 27% of consumers stated that they would agree to pay more than 2F per egg for greater chicken welfare. This analysis agrees with that of many representatives of the food processing industry who anticipate development of the 'alternative' egg market by 2012. The market for free-range eggs should reach 35 – 40% and that of barn eggs should reach 20%
The stakes related to enforcement of stating farming method on labels are high. The definition of labels of origin is likely to enhance a given sector at the expense of another. It must be consistent with the development and communication strategies set up by the egg industry and with certification systems. The absence of technical and economic references related to 'alternative' farming systems compared to conventional cages makes the choice of strategies for conversion of production equipment difficult. Moreover, it is not certain that these systems can maintain the competitiveness of French supply to the lowest price sector, catering and restaurant industries and egg product sectors.

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PRESENT AND FUTURE CONTROL OF FOOD-BORNE PATHOGENS IN POULTRY; REVISION OF THE EUROPEAN COMMUNITY LEGISLATION ON ZOONOSES

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Keywords: preharvest food safety; zoonoses monitoring; *Salmonella*; control programmes

Abstract

Through Council Directive 92/117/EEC, the European Union set up rules for the monitoring of a number of zoonoses and zoonotic agents and required implementation of minimum requirements for the control of *Salmonella* Enteritidis and *Salmonella* Typhimurium in breeding flocks of fowl. The revision of this Directive is one of the actions foreseen in the White Paper on food safety as adopted by the European Commission in January 2000. The new legislation on zoonoses will replace Directive 92/117/EEC and will build on its achievements. With a new Directive, the monitoring of zoonotic agents will be broadened, better co-ordinated and aimed at collecting more comparable data. A new Regulation will focus, initially, on control of salmonella and, possibly in the future, on other agents. Salmonella reduction targets will progressively be set in different categories of animal populations, initially poultry and then pigs.

Résumé

A travers la Directive 92/117/CEE du Conseil, l'Union européenne a établi des règles en matière de surveillance de zoonoses et d'agents zoonotiques et a requis l'application d'exigences minimales pour le contrôle de *Salmonella* Enteritidis et *Salmonella* Typhimurium dans les troupeaux de volaille de reproduction. La révision de cette Directive est une des actions prévues par le Livre Blanc sur la sécurité des aliments adopté par la Commission européenne en janvier 2000. La nouvelle législation remplacera la Directive 92/117/CEE est bâtira sur ses acquis. Avec une nouvelle Directive, la surveillance des agents zoonotiques sera élargie, mieux

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coordonnée et visera à la collecte de données plus comparables. Un nouveau Règlement se concentrera, initialement, sur la maîtrise des salmonelles et éventuellement à l'avenir, d'autres agents. Des cibles de réduction seront progressivement établies dans différentes populations animales, initialement les volailles puis les porcs.

Introduction

The reflection of the Commission on a new approach to food safety resulted in the adoption of the White Paper on food safety in January 2000 (COM (1999) 719 final) that reflected the key policy priority fixed by the Commission in this area. Many actions were announced, in particular the establishment of the European Food Safety Authority and the recast of food safety legislation. Significant numbers have already been finalised or are in the legislative process. Amongst these, a new general food law, Regulation (EC) No 178/2002, has been adopted that covers the whole food chain, including feed production, establishes a high level of consumer health protection and clearly attributes primary responsibility for safe food production to operators. This Regulation also provides the legal basis for the establishment of the European Food Safety Authority (EFSA). The revision of Directive 92/117/EEC concerning measures for protection against specified zoonoses and specific zoonotic agents in animals and products of animal origin in order to prevent outbreaks of food-borne infections and intoxications[1], as amended by Directive 1999/72/EC[2] is part of the programme foreseen in the White Paper on food safety. It implements the principle of a « plough to plate » approach to food safety.

Background for the revision of Directive 92/117/EEC

The review was triggered by the perceived needs to decrease the incidence of zoonoses in humans, to improve the control of zoonoses in the primary production and to strengthen the collection of relevant data to support risk assessment activities and risk management decisions.
The proposals put forward by the Commission concerning monitoring and control of zoonoses are based on the main principles depicted in the White Paper, in particular:
– assuring a high standard of food safety,
– placing the responsibility for food safety primarily upon food businesses, including feed manufacturers and farmers,

[1] OJ L 62, 15.3.1993, p. 38
[2] OJ L 210, 10.08.1999, p. 12

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– ensuring a 'farm to table' policy,
– creating the possibilities for traceability,
– enabling transparency, and
– being based on risk analysis, including the possibilities to take into account the precautionary principle and other legitimate factors, where appropriate.

Review of the epidemiological situation on zoonoses in the EU

In co-operation with the Community Reference Laboratory (CRL) for the Epidemiology of Zoonoses located in Berlin, the Commission services have issued, since 1994, an Annual Report on Trends and Sources of Zoonotic Agents in animals, feeding stuff, food and man in the European Union (and Norway). Recently, the report for 2001 was presented. All countries submitted their annual reports. The quality and quantity of information have increased during the years. However, the data collection usually still suffers from un-harmonised surveillance systems and different quality of contributions, rendering it difficult at present to draw precise conclusions on the trends of zoonotic agents within the EU. Community rules are in force for *Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium (ST) in poultry breeding flocks, which form a basis for comparable data in this sector. The following data are extracted from the report 2001.

Human illnesses

Two zoonoses caused the major part of the reported cases: *Salmonella* and *Campylobacter*, with 157822 and 156232 reported cases in 2001, respectively. These figures are higher than in 2000: increase by 5% for salmonellosis; increase by 1,2 % for campylobacteriosis (10 countries included where figures are available for both years). For salmonellosis, where it had decreased for several years, a decrease was recorded in 2001 in Belgium, France, Ireland, Sweden (and Scotland/Northern Ireland). A few countries recorded large increases. The number of salmonellosis cases exceeds campylobacteriosis cases in Austria, Belgium, Germany and Spain. In contrast, campylobacteriosis was more frequently detected in Denmark, Finland, Greece, Sweden, The Netherlands and United Kingdom.

In the case of other zoonoses, for which information is collected, 10256 cases were reported for *Yersinia*, 1778 for *Brucella*, 860 for *Listeria,* 360 for *Toxoplasma,* 187 for *Echinococcus,* 59 for *Mycobacterium bovis and* 53 for *Trichinella.* The EU report also included information on human cases of verotoxigenic *E.coli* infection, with 2553 cases.

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These figures have to be interpreted carefully, since it is likely that many human infections go unrecorded with patients either failing to present to health services, or no laboratory diagnosis is made, or the diagnosis is not reported centrally. The cases reported may in fact only represent the severe end of the spectrum of the disease.

***Salmonella* and *Campylobacter* in the poultry sector**

- ***Salmonella***

Certain countries run a *Salmonella* control programme approved by the Commission: Austria, Denmark, Finland, France, Ireland, the Netherlands, Norway, Sweden. These programmes vary in particular in relation to the types of animal populations covered (categories of poultry, other species) and to the serotypes covered. While Denmark, Finland, Sweden and Norway provides for control on all *Salmonella* serotypes in fowl, the approved programmes of other countries apply more restricted targets: Austria covers all serotypes in breeding flocks; the Netherlands and to a lesser extent Ireland cover SE/ST in breeders, laying hens and broilers. The French programme includes SE/ST in breeders and laying hens. Some of these countries have programmes in place in other poultry, like turkeys, ducks. The approved programmes of Finland, Sweden and Norway also cover pigs and cattle.

Serotypes in poultry: The attached figures provide the breakdown of *Salmonella* serotypes in the egg production line and the meat production line, at EU level. The data have to be considered with caution as not all countries report or test a representative sample of isolates and the reports do not always cover all serotypes. SE largely dominates in layers (65%). It is also serotype number 1 in broilers (17%).

➢ Breeders

In countries running approved programmes, the situation continued to be very favourable, although there was some increase in one of these countries. The rate ranged between 0 to 4.8 % (all *Salmonella* serotypes included, except France that reported only on SE/ST, with a rate below 2%).
In the other countries, the situation was somewhat different. While in the UK and Belgium, the situation was relatively good (and sometimes improved), the data from Greece, Italy, Portugal and Spain (limited number of samples) showed significantly or considerably higher rates of *Salmonella* infection. For Germany, only some Länder reported.

➢ Layers

Control programmes in layers are run in Denmark, Finland, France, Ireland, the Netherlands, Sweden and Norway (the Nordic countries targeting all serotypes). In Finland and Norway, all flocks were negative for *Salmonella*. In Sweden and Ireland,

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some flocks were infected with serotypes other than SE/ST. In Denmark, the rate reached 4.1% for *Salmonella*, SE being the dominating serotype.

In 2001 as in the previous year, layers usually showed higher rates in comparison to the *Salmonella* situation in the breeding flocks. In France, 2.8% of the flocks in production were found positive for SE/ST. In the Netherlands, the infection rate for SE/ST was higher. In the UK, the number of reports of *Salmonella* decreased. In Germany, with incomplete data, 2.3% of flocks during production were found infected with *Salmonella*. In Greece there was an increase and in Portugal a large decrease in the rate of *Salmonella* positive findings in flocks. In Spain on a limited number of investigations, the rate of *Salmonella* positive flocks was high.

- Broilers

As part of national control programmes, broiler flocks are routinely tested before slaughter in Austria, Denmark, Finland, Sweden and Norway. Other countries also provide data. In 2001, in Finland, Norway and Sweden the *Salmonella* infection rate ranged between 0% and 1%. It was 1.2% in Austria and 1.5% in Denmark (decreasing from 2000). In UK the number of reported *Salmonella* reports decreased. The number also decreased in Germany: 6.8% in 2001. There was no important change in Italy and Portugal with 2.1% and 7.9% respectively. The rate of positive flocks was above 10% in the Netherlands and Greece (small number of investigations).

The contamination rate in poultry meat sampled at slaughterhouses, in cutting plants and at retail tends to reflect *Salmonella* prevalence in broilers in the individual countries, usually with a higher rate of positive tests. For some countries, data were available for both 2000 and 2001. While rates decreased in Austria, Ireland, Germany, Greece, Netherlands, they increased in Italy and Portugal. A majority of countries reported a decrease in their rate, compared to 2000.

- ***Campylobacter***

There is no Community monitoring scheme for *Campylobacter* in animals or products thereof. Some Member States have programmes in place in which they report to the Commission.

Essentially national monitoring programmes on *Campylobacter* in broilers are in place in Denmark, the Netherlands, Sweden and Norway (since 2001), with various schemes that make data impossible to compare between countries. Some testing takes place in Finland. Poultrymeat has been investigated through a screening or continuous programme in the above-mentioned countries and in Belgium and Ireland. Some quantitative data were collected in Denmark and Finland. Some other countries carried out limited investigations in poultry and poultry meat. Based on the information

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received, there is no common trend as regards the prevalence of thermophilic *Campylobacter* in broilers and products thereof. The prevalence in broiler flocks ranged between 4% (Finland; decrease from 2000) to 41.9% in (Denmark; slight increase from 2000) and the rate of positive broiler meat (carcasses **at retail**) ranged between 10 and 30%. While the rate of contamination in poultrymeat decreased in Denmark and Belgium, it increased in Finland and the Netherlands.

The Community legislation regarding the control of zoonotic infections

Implementation of Directive 92/117/EEC and *Salmonella* control

Council Directive 92/117/EEC sought to control *Salmonella* in fowl, with the ultimate goal of eradicating 2 invasive serotypes of *Salmonella* in poultry breeding flocks. It also provided for the development of control measures for other zoonotic agents. A top-down approach was adopted by firstly providing for measures to eradicate SE and ST in breeding flocks to reduce the vertical transmission to commercial flocks. Measures in commercial flocks were foreseen as a possible future step.

Member States should have implemented as from 01.01.1998 the minimum measures laid down for *Salmonella* control in poultry breeding flocks. Most Member States seem to have principally implemented these requirements by 1999.

According to Article 8(2) the Member States should have submitted national plans to control *Salmonella* in fowl before 01.01.1994. However, this time limit was abolished by Directive 97/22/EC pending the review of the Directive, and presentation of *Salmonella* control programmes became optional, although the minimum requirements of the Directive for breeding flocks of *Gallus gallus* must be implemented. The plans from 7 Member States were approved and some of them are subject to Community co-financing of the measures destined to dispose of breeding flocks found infected by SE/ST. These plans go beyond the minimum requirements on breeding flocks of the Directive.

The Commission's Food and Veterinary Office included the implementation of Directive 92/117/EEC when performing on the spot check on poultrymeat production in the Member States a few years ago. Reports are available on the website of DG SANCO (http://europa.eu.int/comm/dg24/health/vi/reports/index_en.html). The missions revealed that not all countries were fully implementing the Directive at that time. The Member States visited that had an approved control programme seemed to

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implement it at least as regards the minimum requirements of the Directive for breeding flocks.

Other legislation

Directive 92/117/EEC is not the only means by which control of zoonoses and zoonotic agents, in particular *Salmonella*, is organised in order to prevent food-borne infections. It requires a concerted multi-disciplinary approach including control measures at all stages of the food chain:
– in animal waste and feed processing,
– at farm level,
– in processing and distribution of foodstuffs of animal origin, and
– at consumer level.

In particular, Regulation (EC) No1774/2002 concerning animal by-products not intended for human consumption contains provisions on *Salmonella*. Microbiological criteria for absence of *Salmonella* have also been laid down in EU legislation for certain products: e.g. for *Salmonella* in milk-based products; egg-products; minced meat and meat preparations. It is worth mentioning that for these products, absence in a certain sample size was decided.

Measures on the control of food borne pathogens in processing and distribution of foodstuffs of animal origin are provided in the corresponding hygiene Directives. These provisions include the ante and post mortem inspection of animals in abattoirs, the requirements for own health checks linked to critical points to be performed by the processors, etc.

Although there are no harmonised criteria for *Salmonella* in poultry meat, it is not exceptional that conflicts in intra-Community trade arise from (repetitive) positive findings in consignments.

New approach towards control and prevention of zoonoses

Specific legislation on zoonoses

Proposals for a revision of Council Directive 92/117/EEC were adopted by the European Commission on 1 August 2001 and submitted to the Council and the European Parliament for their co-decision. The revised legislation will comprise two pieces of legislation that will repeal and replace Directive 92/117/EEC:
– a Parliament and Council Directive on the monitoring of zoonoses and zoonotic agents and

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– a Parliament and Council Regulation on the control of *Salmonella* and other specified food-borne zoonotic agents.

The Directive

A critical issue for the optimal implementation of the Community strategy on microbiological food safety, will be the collection of epidemiological/scientific data on zoonoses. It is one of the tasks identified for the EFSA. In the future the measures intended to combat zoonoses should be based on risk assessments. This will increase the importance of accurate information on zoonoses and zoonotic agents from all stages of the food chain. Surveillance will cover the whole food chain and all types food (whether it is of animal of non-animal origin, following a change introduced on request of the European Parliament). Monitoring will be based on the systems in place in the Member States, but efforts should be directed to harmonise as appropriate the reporting systems in the Member States to ensure significance and comparability of data, which was an objective stressed in particular in the European Parliament. The different authorities with competence in feed, animal, food, human area will be required to cooperate. Co-ordinated monitoring programmes may be needed to establish e.g. base-line values, which could be used as starting prevalences for the pathogen reduction foreseen in the Regulation. Monitoring of anti-microbial resistance in *Salmonella* and *Campylobacter* (and possibly other relevant agents in the future) and investigation of food-borne outbreaks will be emphasised. The European Food Safety Authority (EFSA) will be involved in the scientific consultations and will produce the Community report on zoonoses monitoring. The data in humans will be collected through the Community Communicable Diseases Network and data should be made available to EFSA.

The Regulation

The purpose is to ensure that proper and effective measures are taken to detect and to control *Salmonella* and other zoonotic agents, particularly at the level of primary production, in order to reduce their prevalence and the risk they pose to public health. It creates a framework for zoonoses control and control measures will be defined more closely by further decisions.

The main elements are as follows:
– A procedure is established to set targets to reduce the prevalence of specified pathogens at different steps in the foodchain, within a defined period. The emphasis is placed on objectives to be reached, leaving a certain level of flexibility to the Member States to decide on the measures to reach the objectives.

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– *Salmonella* is the primary zoonotic agent targeted at primary production as it represents an important burden to public health and the new measures to control *Salmonella* can be built on the measures initiated under Directive 92/117/EEC. Furthermore, experience shows that controls at primary production can be efficient.

– Targets for other zoonoses and/or other steps in the food chain can also be set, if appropriate. The Community Economic and Social Committee, a small number of Member States and at a preliminary stage the European Parliament, considered that other zoonotic agents like *Campylobacter* should be included. The opinion of the European Commission's Scientific Committee on veterinary measures relating to public health (SCVPH) on 'food-borne zoonoses', issued on 12 April 2000, indicated that there was still a lack of knowledge on the epidemiology of the agent and on the feasibility and efficiency of controls in particular at primary production (opinion available on: http://europa.eu.int/comm/food/fs/sc/scv/out32_en.pdf). It was agreed not to include targets for this agent.

– Poultry and eggs, followed by pork are considered to be the main sources of food borne salmonellosis in humans. According to the proposal from the Commission, the *Salmonella* targets are to be set for different categories of poultry (*Gallus gallus* followed by turkeys) and pigs (slaughter pigs and breeding pigs), on a step-by-step basis. Targets on different animal populations will be set usually every year: the first target will be established for breeding flocks of *Gallus gallus* 12 months after entry into force of the Regulation, for laying hens one year later, for broilers one year later, for turkeys (and pigs) one year later. It was agreed with the Council and the European Parliament not to include other animal populations than poultry and pigs at this stage.

– The targets should include *Salmonella* with public health significance and certain criteria are set how to identify the relevant serotypes. In that framework, the Commission before proposing reduction targets will be required to perform cost/benefit analyses. Transitional periods have been established for the targets on poultry so that during the first 3 years for each relevant animal population, the number of serotypes will be restricted.

– The Member States should prepare national control programmes in order to meet these targets, and the Commission will approve those programmes. The programmes shall be approved by the Commission and enter in force within 18 months after the corresponding target is set. The food businesses could establish their own control programmes that may be recognised by the national authorities as part of the national programme submitted to the Commission.

– Minimum sampling requirements in animal populations are set in the Regulation.

– Certain pre-fixed benchmarks are set in the Regulation. Within 30 months after programmes are in operation on laying hens, table eggs shall come from flocks subject to a national programme and not under restrictions; and within 30 months after programmes are in operation on broilers, poultry meat containing *Salmonella* in 25g

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shall not be placed on the market other than for an industrial treatment able to eliminate *Salmonella*.

– The certificates for trade in relevant live animals and hatching eggs will be amended in due course to include a statement concerning the status of the flock or herd of origin. Certain additional guarantees may be granted for a temporary period.

– Equivalent guarantees will be requested for imports from non-member countries.

– Last but not least, the Commission agreed to consider eligible for Community co-financing, the mandatory control measures that will be progressively introduced pursuant to the new Regulation.

Other legislation

The Community legislation on food hygiene is currently being restructured and revised in order to establish a coherent and consistent body of hygiene rules based on an integrated approach covering the whole food chain "from stable to table" (COM(2000) 438 final). The new legal instrument on food hygiene will cover the entire production chain of all foodstuffs, both of animal and of plant origin. One of the main objectives of the new legislation is to prevent food-borne infections. Efforts will be directed to highlight the producers' liability in ensuring the food safety and to establish HACCP-type principles in controls. This principle is not intended to cover the primary agricultural production, but measures to control hazards will have to be implemented. A general obligation will be established providing for the Member States to ensure that Good Hygiene/Farming Practices (GHP) are complied with in livestock production. Guides to GHP should be developed. This will be essential to prevent contamination and spread of zoonotic agents in farms. Enhanced records will have to be kept by operators and relevant information will have to follow animals going for slaughter.

As another element of food-borne pathogen control, in the context of the revised EU food hygiene legislation, the Commission is in the process of revising the EU microbiological standards. The usefulness and relevance of *Salmonella* criteria will be considered in that framework.

Conclusions

The provisions of Directive 92/117/EEC needed to be revised in the light of experiences gained in their implementation. Poultry products still form a major source of human salmonellosis and represent a serious public health burden (according to a rough estimate, the health cost of food-borne zoonoses in the EU was MEURO 620-

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3160; cf COM(2001) 452 final). Nevertheless, the results of risk assessment may in future reveal other important sources of zoonotic agents or new types of organisms may emerge. Therefore a framework for control of zoonoses must be established.

It is clear from the above that establishing a long-term strategy for the monitoring and control of zoonoses is a complicated issue, for which many aspects must be taken into account. Apart from the paramount objective of public health and consumer protection, aspects such as scientific assessments, practicability of the proposed measures, follow-up of the efficiency of pathogen reduction programmes and the financial impact of such measures play a role in any future policy for controlling zoonoses.

The new approach is intended to further improve the safety of consumers especially having regard to the pathogens with the greatest importance to health. At the same time consumers have to accept that a zero level risk is not usually achievable nor even feasible.

Last but not least, a risk-based Community policy to control zoonoses and zoonotic agents would also help the Community to defend its position in relation to trade with third countries.

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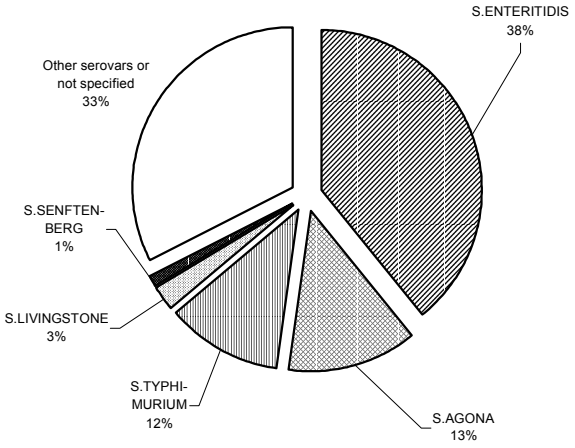
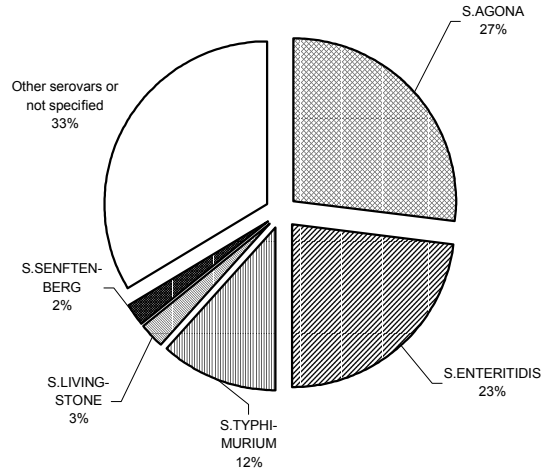
The five most frequent *Salmonella* serovars (in %) in the meat and egg production lines, 2001

Broiler Breeder



Layer Breeder



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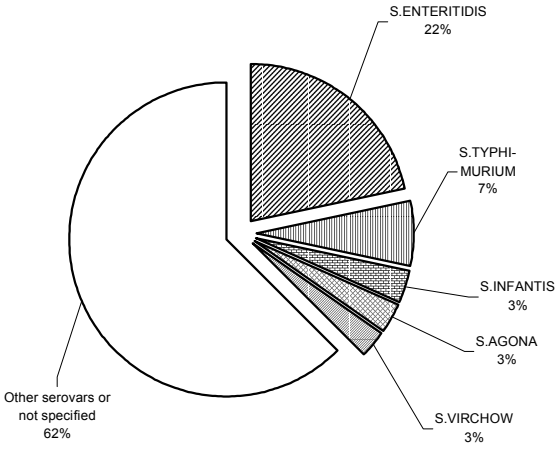
Broiler



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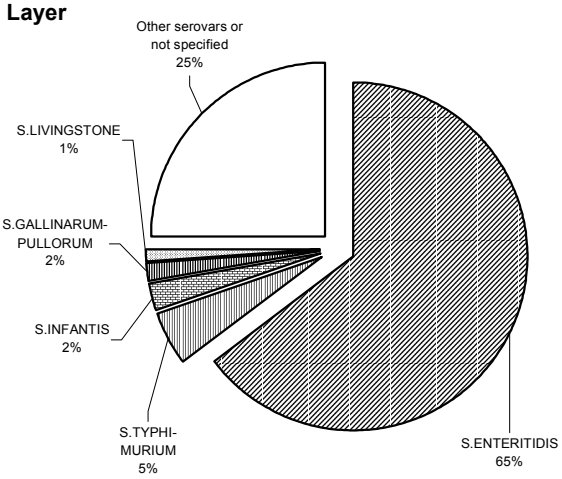

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Session 2
Hygiene and microbiology of poultry production

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CURRENT TRENDS IN THE MICROBIOLOGICAL SAFETY OF POULTRY MEAT

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Keywords: poultry, *Salmonella*, *Campylobacter*, production, processing

Abstract

Contamination of poultry meat products with *Salmonella* and *Campylobacter* spp. continues to be the main microbiological problem affecting both industry and consumers. In some countries, control measures appear to have reduced flock infection with *Salmonella*, but other issues have arisen; these include an increase in multiple resistance to antimicrobials in certain serotypes and the recent emergence of *S.* Paratyphi B variant Java, also frequently multi-resistant. For intensive production systems, there is much information on sources and routes of *Salmonella* transmission, and the relative risk of infection / contamination at different stages of production. Some possible reasons for control failures are discussed. In contrast, less is known about *Campylobacter* in these respects, and a better understanding is needed of the physiology and host-interactions of the organisms, and of their behaviour in poultry operations. On such knowledge may depend the future development of effective controls.

Résumé

La contamination des viandes de volailles par *Campylobacter* et *Salmonella* constitue une source d'inquiétude pour les industriels comme pour les consommateurs. La mise en place de moyens de lutte dans certains pays a permis de réduire le nombre de paquets positifs en Salmonella, mais ne les a pas mis à l'abris de l'apparition de sérotypes multirésistants aux antibiotiques ou de l'émergence récente de S. Paratyphi B variant java, parfois multirésitante aux antibiotiques. Les facteurs de risques et voies de contamination par Salmonella des filières avicoles intensives sont bien connues aux différents stades de la production. Les possibles causes de disfonctionnement des mesures de maîtrise seront discutées. Au contraire, peu d'informations sont disponibles sur la contamination de la filière avicole par Campylobacter, et les

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connaissances doivent être développées en matière d'interaction hôte-microorganisme et sur le comportement et la survie de Campylobacter au cours des opérations de productions et de transformation de volailles. Le développement ultérieur de mesures efficaces dépend de l'acquisition de ces connaissances fondamentales.

Introduction

Despite the considerable efforts made in many countries to reduce the incidence of foodborne illness, poultry meat remains an important vehicle, especially in relation to certain serotypes of *Salmonella enterica* and thermophilic *Campylobacter* spp. Part of the reason for this is that either kind of organism may be carried asymptomatically in the alimentary tract of the live bird and then spread via the slaughter process to raw, finished products. Subsequent consumption of undercooked or recontaminated poultry meat, or the handling of raw poultry are major risk factors in human infection (Bryan and Doyle, 1995). For both *Salmonella* and *Campylobacter jejuni / coli*, the true proportion of human cases associated with poultry is unknown, largely because many incidents are sporadic, ie single cases, and cannot be identified with the consumption of a particular food item. Most of the information that is available on possible vehicles comes from outbreak data and a few case-control studies (Report, 1996). Although the position will vary from one country to another, data for England and Wales show that between 1992 and 1999, a fifth of all general outbreaks of infectious intestinal disease that were reported to the public health authorities could be linked to poultry consumption (Kessel *et al*., 2001). Of these, 60% were due to *Salmonella*, 21% to *Clostridium perfringens* and 6% to *Campylobacter* spp. In total, there were 7000 affected individuals, with 258 admissions to hospital and 17 deaths. For *Salmonella* alone, previous estimates from the literature showed that poultry was likely to be responsible for 20 – 35% of all reported cases (Report, 1996). With regard to *Campylobacter*, which is not commonly involved in outbreaks,indirect evidence for the role of poultry consumption was obtained in Belgium. Due to dioxin-contamination of feed, poultry meat and eggs were withdrawn from sale and this resulted in a 40% decrease in reported cases of *Campylobacter* infection (Vellinga and Van Loock, 2002).

The most recent surveillance data show that human salmonellosis has decreased substantially across the European Union (EU), the number of reported cases declining from a peak of 100,267 in 1997 to 73,006 in 2001 (O'Brien and de Valk, 2003). Only Spain has continued to report high rates of *Salmonella* infection, partly due to continuing contamination of table eggs with *S.* Enteritidis.

By contrast, the *Campylobacter* situation has been more variable, with some countries experiencing a steady increase in cases over the last 20 years and others showing a

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more recent decline. In England and Wales over the last five years, there have been consistently around 50,000 reported cases annually, which could equate to an actual figure of *ca* 0.5 million affected individuals.

Although the recent reductions in human salmonellosis are encouraging, and appear to be partly the result of improved control measures in the Poultry Industry, especially in relation to *S.* Enteritidis, new challenges are emerging. In the Netherlands, for example, *S.* Paratyphi B variant Java has increased in poultry from less than 2% of isolates before 1996 to 60% in 2002 (van Pelt *et al*., 2003). As yet, there have been few human cases from the organism, but it has also appeared in Germany and Scotland and, in some instances, molecular subtyping has shown a clear link between poultry and human infection, which is associated with international trade. Variant Java is unusually persistent in the poultry rearing environment and, increasingly, isolates show resistance to one or more antimicrobials, including quinolones. In fact, most strains are multi-resistant. With salmonellas in general, this is a growing problem, and the testing of 27,000 isolates from human cases in 10 European countries in 2000 showed that almost 40% were resistant to at least one antimicrobial, while 18% were multi-resistant, including serotypes often associated with poultry (Threlfall *et al*., 2003). Resistance to ampicillin, streptomycin, sulphonamides and tetracycline was common. In all countries, multiple resistance was most often observed in serotype Typhimurium, including DTs 104 and 204b, and 51% of Typhimurium strains were in this category. With strains from England and Wales, multiple resistance was also prevalent in *S.* Hadar and *S.* Virchow, while in other countries it occurred in different serotypes. The resistance of *Campylobacter* to antimicrobials is also reported to be increasing (Report, 2001). The most alarming increase is in resistance to fluoroquinolones, which are used in both human and veterinary medicine and are widely employed in poultry production.

Contamination of processed products

Generally, there are differences between *Salmonella* and *Campylobacter* in the proportion of carcasses in any one batch that become contaminated, and in the numbers of organisms present on positive carcasses. Both are usually greater for *Campylobacter* and reflect different levels of intestinal carriage at slaughter. Also, surveys have shown that up to 90% of poultry flocks can be colonised with *Campylobacter*. Most salmonellas are poorer colonisers and are shed in relatively low numbers, especially as birds grow older (Poppe, 2000). On the other hand, *C. jejuni* may be present in slaughter-age broilers at levels up to 10^9 cfu / g of intestinal contents (reviewed by Mead, 2002). Both types of bacteria include strains that are invasive in poultry and may therefore be present in internal organs or deep tissues. A

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review of the literature on contamination of carcasses and other products was made by Waldroup (1996). Data from 18 countries show how widely the proportion of salmonella-positive carcasses can vary, but counts rarely exceeded 100 cfu / carcass, whereas campylobacters often numbered thousands or millions per carcass. Campylobacter contamination occurs over the entire carcass surface and Berndtson *et al*. (1992) reported that occasional muscle samples were also positive.

In the slaughterhouse, *Salmonella* is very much a minority organism that may be present in relatively few of the in-coming birds, but is likely to spread to previously uncontaminated carcasses during processing. Cross-contamination will also occur with *Campylobacter*, and is important in relation to flock-to-flock transmission. It could be significant within an infected flock, but at present, it has to be assumed that all strains have the same potential to cause human illness. Thus, if 'harmless' strains exist, they cannot be distinguished from any others. As carcasses pass through the different stages of processing, the overall burden of *Campylobacter* contamination will be reduced, especially at the earlier stages, where feathers, viscera and other parts of the bird are removed and the organisms are washed off carcass surfaces during scalding and spray-cleaning. However, the extent of the reduction depends upon processing practices. When processing involved the use of super-chlorinated water for spray-washing carcasses and selected items of equipment, followed by chilling of carcasses in chlorinated water, Mead *et al*. (1995) showed a mean reduction of about one hundred-fold. Counts of *Campylobacter* from neck-skin samples decreased from an initial log 3.7 cfu / g to 1.8 cfu / g. The effect was similar to that reported by Waldroup *et al*. (1992), who modified the process to include counterflow scalding, addition of a post-scalding spray-washer and appropriate chlorination of all water used for carcass washing and chilling. Even better results could be expected with the use of a separate carcass decontamination treatment, as discussed by Hinton and Corry (1999). In the EU, super-chlorination of process water has not been accepted and its use in the UK has been discontinued. Furthermore, water immersion chilling is more rarely used now in the major European poultry-producing countries and, as the market has moved further towards chilled rather than frozen products, air-blast chilling has become almost universal. The process is often used in conjunction with fine water-sprays to provide evaporative cooling.

Properly controlled immersion chilling, either with or without super-chlorinated water, can be expected to reduce carcass contamination by about one log unit, but has no beneficial effect on the proportion of *Salmonella*-positive carcasses in a particular batch (Mead, 1989) and the situation is likely to be similar for *Campylobacter* (Berrang and Dickens, 2000). Although *Campylobacter* is relatively sensitive to chlorine, when suspended in an aqueous solution, resistance is greatly increased by attachment of the organisms to carcass skin (Mead, unpublished data). Modern air chilling, on the other hand, has no washing effect that is comparable to immersion chilling and any influence on *Campylobacter* may be due largely to drying of carcass

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surfaces. However, carcasses do not dry evenly or sufficiently well to eliminate *Campylobacter*, even when chilling times are extended (Mead and Hudson, 1987). Nevertheless, further developments in air chilling may be able to exploit the sensitivity of campylobacters to drying treatments, and this aspect is currently being investigated.

Reducing product contamination

Past food 'scares', greater producer liability and the expectation of consumers that the food they buy will be essentially risk-free have helped to focus attention on the need to minimise contamination of raw poultry with foodborne pathogens. The desirability of this goal is reflected in various national strategies. In 2000, the UK Food Standards Agency (FSA) set a target to reduce *Salmonella* contamination of retail chicken produced in the UK by 50% within five years. Efforts involved in meeting this target include the identification of necessary improvements in industry practices. Already, the overall frequency of *Salmonella* contamination is down to 5.7%, the lowest figure for at least 20years (FSA data). In The Netherlands, the broiler sector aims to reduce the proportion of *Salmonella*-positive flocks to 5% by January 2004, as judged by sampling processed carcasses. From 1998 onwards, the US Food Safety and Inspection Service has been operating a Pathogen Reduction Programme which involves the enforcement of performance standards for *Salmonella*-contaminated carcasses. In the case of broilers, the initial target figure was 20%, reducing to lower levels as performance improved according to the results of national surveys (USDA, 1996). Although it is accepted universally that process hygiene is important in pathogen control, it is also evident that product contamination has its origins in live-bird production and, therefore, increasing attention is being given to the improvement of on-farm control and possible development of new intervention measures. In practice, controls are needed at all stages of the poultry supply chain, involving, ideally, a fully integrated approach by each company, in which the application of Hazard Analysis Critical Control Point (HACCP) principles is seen as a key part (Report, 1996).

Role of production factors

Considerable variation occurs in the geographical distribution of particular *Salmonella* serotypes in poultry, and in their individual behaviour and persistence in poultry operations. The main sources of flock infection were reviewed by Poppe (2000) and include vertical transmission, contaminated feed and a variety of possible vectors in

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the rearing environment. In practice, many of the factors that affect vertical and horizontal transmission are interrelated. Also, the relative importance of the different sources can vary from one commercial situation to another. Whereas some studies have shown that *Salmonella* contamination of feed ingredients can pass all the way through the poultry production chain and cause illness in humans (Williams, 1981), in other instances this has not been the case. More recently, an epidemiological study by Heyndrickx *et al*. (2002) found no correlation between salmonellas in live poultry and contamination of the end-product, indicating that contamination 'hot spots' were occurring in the processing plant. Despite these differences, however, it can be argued that the requirements for *Salmonella* control in the Poultry Industry are well-enough known for effective control programmes to be implemented at all stages of the supply chain and, among many poultry producers, such controls are already in place.

To further improve the situation, a number of other measures could be taken, especially in relation to rearing of broilers, and examples are as follows. A potentially important factor is the *Salmonella* status of poultry feed. In the UK, feed contamination appears to be at a low level (Report, 2001) and some of the serotypes present are not found in poultry flocks to which the feed is given. Nevertheless, the UK Advisory Committee on the Microbiological Safety of Food suggested that feed could, and should, be *Salmonella*-free (Report, 1996). In order to achieve this aim, the Committee recommended closer adherence to existing guidelines on all aspects of feed production and a phased programme of investment to upgrade feed mills and associated facilities. In the meantime, improvements in the process have been made in the UK, HACCP principles have been introduced and selection of raw feed ingredients has become more rigorous, but salmonellas have not yet been eliminated. Even with adequate heat treatment, there are still opportunities for re-contamination during cooling, handling, storage and distribution, and in some mills, at least, more effective hygiene control would be beneficial.

By intensifying the microbiological monitoring of broiler houses after routine cleaning and disinfection, it has become apparent that residual salmonellas often remain in low numbers from a previously infected flock (Wray *et al*., 1999). The problem is exacerbated in older housing, where effective cleansing is more difficult, and farms of this kind often have a site layout that is not conducive to good biosecurity. In some operations, the cleaning and sanitising process needs to be critically appraised, monitored more thoroughly and improved. Even without such an improvement, there would be good reasons to use available intervention measures such as acid-treatment of feed or 'competitive exclusion' (CE), but, in most countries, these are applied only sparingly, if at all, to broilers, often because of cost. Enhancing flock-resistance to *Salmonella* colonisation by early establishment of an adult-type intestinal microflora would be particularly relevant to broilers. This is because of the slow rate at which the natural flora develops and the need to avoid any period of *Salmonella* shedding that might contaminate the skin and feathers of the birds. Lastly, there is growing

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recognition of the role of transport crates in spreading *Salmonella* (and *Campylobacter*) contamination from previous flocks among birds being taken for slaughter. Reasons why crates remain contaminated after being cleaned at the processing plant are discussed by Corry *et al*. (2002).

In contrast to *Salmonella*, much less is known about the actual sources and modes of transmission of *Campylobacter*. Present knowledge of similarities and differences between the two organisms is shown in Table 1. Whereas *Salmonella* is more of a problem in young birds, flocks kept in houses are rarely colonised by *Campylobacter* before two or three weeks of age. It has been suggested that maternal antibodies may be responsible for the initial period of apparent resistance (Sahin *et al*., 2001), but more detailed information is needed on possible risk factors for flock infection and on bird colonisation by *Campylobacter* cells that may be dormant or sub-lethally injured following environmental exposure. The more rapid onset of infection that can be observed in flocks reared out-of-doors (Dr V M Allen, personal communication) could be due to the presence of a larger or more active challenge under these conditions. Although there is some evidence that campylobacters can be transmitted vertically (Pearson *et al*., 1996; Cox *et al*., 1999), horizontal transmission from a range of potential vectors or sources seems more likely in most circumstances and is more obviously in keeping with the delay in bird colonisation. Unlike the situation with *Salmonella*, however, manufactured feed appears to be too dry to allow survival of campylobacters. Supplies of potable water would also seem to present little risk of introducing the organisms, but some unchlorinated borehole waters could be contaminated, and special attention is needed to the cleaning and disinfection of the water distribution system inside the house, since campylobacters from a previous flock can survive well in any biofilms formed. Apart from maintaining high standards of biosecurity, including a hygiene barrier, there are no commercially available intervention measures that have any marked effect on *Campylobacter*. Current research is aimed at finding some means of reducing levels of intestinal carriage in the bird, eg bacteriophage treatment. Any approach to developing a vaccine would need to target colonisation factors, which are poorly understood at present. Available CE products are effective against *Salmonella*, provided that chicks are initially *Salmonella*-free (Deruyttere *et al*., 1997), but have little effect on *Campylobacter* under field conditions. Because specific intervention measures are lacking, many companies are reluctant to carry out regular monitoring of either broiler flocks or processed carcasses; however, testing would enable some flocks to be re-scheduled for slaughter to avoid cross-contamination. In the longer term, a better understanding is needed of the physiology and host interactions of campylobacters and of their behaviour in poultry operations. Such knowledge may well be necessary for the future development of effective, practicable control measures.

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Table 1 Transmission and control potential compared for *Salmonella* serotypes and thermophilic *Campylobacter* spp.

Characteristic	Salmonella	Campylobacter
Mainly young birds susceptible	+	-
Potential for vertical transmission	+	+
Environmental persistence	+	?*
Possible horizontal transmission from:		
feed	+	-
rodents	+	+
wild birds	+	+
insects	+	+
contaminated water supply	+	+
external environment via footwear of stockman	+	+
Availability of vaccines, competitive exclusion treatment	+	-

* Apparently poorer survival than *Salmonella,* but existence of viable, non-culturable forms.

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EGG PRODUCT PRESERVATION PROCESSES: A REVIEW

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Keywords: Egg products, functionality, contamination, preservation processes

Abstract

Eggs and egg products are considered to be the major sources of confirmed food poisoning. In order to ensure compliance to microbial standards, several technological means can be considered for egg products, still based on microorganism separation, inhibition, or destruction. Microorganism separation could be achieved by microfiltration or centrifugation, but is difficult because of shear stress sensitiveness and viscosity of egg products. Microorganism inhibition is usually implemented by freezing, drying, or the addition of sugar and salt. Concerning microorganism destruction, heat treatment is widely used for liquid egg products and dried egg white. Heat treatment of liquid egg white is kept mild enough to limit denaturation of its thermally unstable constituents and does not result in systematic conformity to microbiological standards. Non-thermal processes are conceivable for egg product preservation, but leads to technological and consumer expectation problems.

Résumé

Les œufs et les ovo produits comptent parmi les principales œuvres de toxi-infections alimentaires. Afin d'assurer la conformité des ovo produits aux critères micro biologiques, plusieurs technologies faisant appel à la séparation, l'inhibition ou la destruction des micro organismes, peuvent être envisagées. La séparation des micro organismes par micro filtration ou centrifugation peut être envisagée, mais reste délicate compte tenu des forces de cisaillement qu'elle entraîne et de la viscosité de l'œuf. L'inhibition de la croissance des micro organismes peut être atteinte par congélation, séchage ou addition de sel ou de sucre. La pasteurisation est largement utilisée pour la destruction de micro organismes dans l'œuf entier liquide ou le blanc déshydraté. Le traitement thermique du blanc liquide doit rester modéré pour préserver les propriétés physiques et en conséquence conduit parfois à l'obtention des produits non conformes micro biologiquement. Les procédés non thermiques sont

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envisageables, mais conduisent à des problèmes technologiques ou à un rejet des consommateurs.

Introduction

The increase of egg product processing was about 77% from 1990 to 1999 in France (Magdelaine & Mirabito, 2000). Presently, approximately 25% of the French egg production is transformed into egg products, including 25% of egg white, 15% of egg yolk and 60 % of whole egg. These products (liquid, dried or frozen) are widely used in the manufacturing of various foodstuffs for their functional properties, and good microbiological quality is essential, especially when they are used in the preparation of uncooked food products. At the same time, eggs and egg products are considered to be the major sources of confirmed *Salmonella* toxi-infections in France (Haeghebaert et al., 2001). The monitering and eventual optimization of the preservation processes represents, therefore, the main key point for the development of the egg product sector, for which France is a European leader. Our purpose is here to focus on these processes. The characteristics of both composition and contamination of egg products will be first recalled. Following this, the various processes will be presented, according to the principle of their action on the micro-organisms: separation, inhibition, or destruction. We will discuss more particularly about egg white processing; indeed, yolk and whole egg processing is not so difficult, with regard to their sensitivity to heating and shearing.

Composition and contamination of egg products

From a biochemical point of view, egg product composition includes glucose, minerals and proteins (egg white) and/or lipids and lipoproteins (egg yolk and whole egg). Glucose is known to be a reducing sugar, and thus can interact with amine groups of peptides and proteins (Maillard reaction). This condensation, occurring during heating and drying, results in the formation of a brownish color and reduction of the nutritive value. This explains why glucose is often removed from the liquid prior to drying in order to produce a stable egg product. Egg white proteins are known for their functional and biological properties (*Table 1*). Some of these proteins have a high sensitivity to shearing (ovomucin, ovalbumin) and heating (ovotransferin), connected to the functional properties of the egg white. These constitute a limiting factor of the implementation of technological treatment resulting in high temperature values or high

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shear stress (heat treatment or filtration). On the other hand, most of these proteins have an antimicrobial effect, which prevents microbial development in eggs product.
From a microbial point of view, egg white presents a low iron concentration, an alkaline pH and a large number of proteins inhibiting the growth of microorganisms, while egg yolk and whole egg constitute favorable media for bacterial growth. Shell eggs are in general sterile, except in the case of transovarian contamination by *Salmonella*. Egg breaking results systematically in egg white and yolk contamination by contact with soiled shells (from hen cloaca or environment), thus leading to very perishable products. Microorganisms may also come from the factory environment (surface, machine, water, air) or from operators. Many pathogenic (*Salmonella*, *Bacillus cereus*, *Staphylococcus*, *Clostridium*) or spoilage micro-organisms (*Pseudomonas*, *E. coli*, *Enterococcus*, *Streptococcus*, lactic flora, yeast and moulds) can thus contaminate egg products (Lock & Board, 1992). The contamination by *Salmonella* remains the major safety problem of the sector: in 1998, it represented 71% of the food poisoning cases when the microorganism involved was identified, shell eggs and egg products counting for 81% of these food poisoning cases (Haeghebaert et al., 2001).

Microorganism separation processes

These processes are based on a nondestructive separation of the micro-organisms while exploiting their physical characteristics (size, density). They consist mainly of tangential microfiltration and centrifugation.
Tangential microfiltration is carried out with membranes of pore diameter ranging between 10^{-7} and 10^{-5} m. It allows the selective concentration of the dispersed elements of the treated liquid, among which micro-organisms are included. For this reason, it can be implemented to make microbial epuration to replace heat treatment, and makes it possible to usually reduce by 3 to 4 log the initial population. However, the studies carried out to date with egg white highlighted a technological bolt related to the mechanical denaturation of proteins during their transmission through the membrane, where they are subjected simultaneously to a considerable increase in interface (10^6 $m^2.m^{-3}$ of product) and to high shearing forces. The denaturation of proteins, especially of ovomucin, in the membrane pores results in their irreversible blocking. In order to overcome this difficulty, which determines the technical feasibility of this operation, ovomucin could be separated from the egg white before microfiltration, using a 3 fold dilution, lowering of the pH from 9 to 6 and holding these conditions for 24 hours at 4°C. However, this operation is not conceivable for mass production and for significant volumes, taking into account its economic and environmental consequences.

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Centrifugation consists of decantation under a centrifugal acceleration field. It is often carried out in the dairy industry for the separation of butyric spores (heavy phase) from raw milk (light phase). The decantation speed v_c is given by the Stokes law:

$$v_c = \frac{D^2}{18.\eta}.(\rho_p - \rho_f).\omega^2.R$$

in which D is the micro-organisms diameter, η is the viscosity of the dispersing phase, ρ_P and ρ_F are respectively the densities of the micro-organisms and of the fluid, ω the angular velocity and *R* is the distance to the rotation axis. Centrifugation is a way to eliminate the yeast cells (*Saccharomyces cerevisae*), sometimes added to the egg white in order to remove glucose before drying. In this case, the yeast diameter is such (10 to 30 μm) that separation can be obtained at moderate angular velocity. However, and except in that case, the centrifugation of egg products is not practised. Indeed, the viscosity of egg white is 3 to 10 times higher than that of milk, which would imply increasing angular velocities up to 6000 $rad.s^{-1}$, that is to say 6.10^4 turns per minute. This is obviously unrealistic without specialist equipment.

Microorganism inhibition processes

The inhibition of micro-organisms consists in lowering the rate of their kinetics of growth k_θ by lowering temperature, pH or water activity a_w, or the addition of inhibitors. The **refrigeration** consists in lowering the temperature below 10°C (reference temperature: 4°C). Within this range of temperature, the generation time of the microorganisms becomes almost infinitely long. Indeed, the relationship between k_θ and the temperature is given by the Arrhenius law :

$$k_\theta = a.e^{\frac{-E}{R.T}}$$

In which a is a constant, E the energy of activation of the reaction, R the constant of the gas law and T the absolute temperature. Taking into account the range of energies of activation of the bacterial growth, a decrease of 10°C in temperature makes it possible to divide k_θ by 2. On the other hand, bacteria toxin production is stopped at low temperatures. The refrigeration thus makes it possible, simultaneously, to limit the kinetics of bacterial growth (as an example, the minimal growth temperature is 6,7°C for *Salmonella*) and the production of toxins. The **lowering of pH** below the isoelectric point of proteins could be proposed. In general, none of the microorganisms previously mentioned is able to develop below pH 4.5. In the case of egg white, the basic pH is close to the limit of bacterial growth, and an increase in pH could be also a

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solution to prevent egg product spoilage. However, none of these processes are used on a mass production scale, because of lack of user demand for such products.

The **decrease of a_w** is obtained by the reduction of the water content (concentration and drying) or water mobilization (congelation or addition of hydrophilic molecules).

The **concentration** of the egg products before drying is useless in the case of the yolk and whole egg, taking into account their high level of dry matter. On the other hand, the egg white must be concentrated to 20% of dry matter before drying to obtain satisfactory spraying conditions. Although some authors report the use of vacuum evaporation at this stage, this process is not currently practiced. Indeed, it would be necessary to maintain pressure lower or equal to 0,16 bar in order to be located below the range of thermal protein denaturation, corresponding in practice to no more than 2 stage installations. On the other hand, the use of this technology results in the significant formation of foam and a deterioration of functional properties of egg white, which can be related to the expansion of the product coupled with high shearing forces. This is why the concentration of egg white is usually carried out by tangential filtration, ultrafiltration or reverse osmosis. Contrary to microfiltration, these processes do not raise major difficulties, because in this case the proteins do not pass through the membrane and are integrally concentrated.

The **drying** of egg products is mainly carried out in horizontal towers, where the product is atomized by spray nozzles in fine droplets and mixed with the hot air in co-current flow. The surface thus created is considerable (1 to 3.10^5 $m^2.m^{-3}$ of the product), which allows evaporation and water transfer from droplets to air in less than one second. During drying, the temperature of the product increases from the wet bulb temperature of incoming air (≤ 50°C) to the temperature of outlet air at the end of the drying (≤ 75°C). The powder moisture ranges of 7 to 9%, corresponding to an a_w of between 0,2 and 0,3. Drying affects functional and organoleptic properties of the egg white. The reduction of the whipping capacity of the dried egg white, compared to the liquid egg white, is related to the surface denaturation of proteins due to significant shearing and to the increase of interface during atomization. The heat treatment to which the product is subjected during drying is not expected to be involved in this denaturation, because of the protein thermal sensitiveness decreases with a_w. On the other hand and in the case of egg white, it has a direct consequence on the development of a brown color (Maillard reaction) between glucose and the proteins, which results in a cooked flavor. The removal of glucose before drying makes it possible to overcome these difficulties.

Freezing also makes it possible to stabilize the egg products by decreasing the a_w (the water being present in a crystalline structure) and of reaction kinetics according to the Arrhenius law (bacteriostatic effect). In addition, a special bactericidal effect occurs in the temperature range in which ice crystallizes out (from -0.55°C to -5°C for egg products), and a reduction from 1 to 2 log of the microbial population is usually observed. This would be due to cellular membrane deterioration by ice crystals. The

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lifespan of frozen egg products, which account for 10% of French production, is approximately 24 months. The quality of the egg white after defrosting depends primarily on good control of the freezing-defrosting process: fast freezing which leads to small size ice crystals doesn't modify the characteristics of the egg white, while a slow freezing (large crystals) led to a defrosted egg white partially coagulated. Upon freezing and storing raw egg yolk below –6°C, the viscosity increases and gelation occurs. These changes are considered to be partially irreversible. Lastly, freezing and cold storage can select for a psychrotrophic flora, and some increase in the number of bacteria can occur upon slow defrosting.

Sucrose or sodium chloride addition (approximately 10%) is also realized, according to the destination of the products. Apart from their organoleptic impact, these additions also contribute to a reduction of the a_w and thus result in a better conservation of the product. Moreover, these additive allow the increase of the heat treatment applied and reduce the effect of other treatments on the components and functional properties of the egg products (drying, congelation). Lastly, the addition of bacterial inhibitors such as sorbic acid(E 200) or benzoic acid (E 210), as well as their associated bases (e.g. sodium benzoate or sorbate), is authorized for the liquid egg products at a rate of 5 $g.kg^{-1}$.

Microorganism destruction processes

Microorganism destruction is carried out by heat treatment of egg products. Taking into account the thermal sensitiveness of their proteins (*Table 1*), the treatments applied to liquid egg products are weak and allow only the destruction of non spore bearing bacteria: this corresponds thus to pasteurization treatment. The destruction of micro-organisms at a constant temperature θ is well described by means of first order reactions, whose integration leads to the equation:

$$\log \frac{N_0}{N} = \frac{t}{D_\theta}$$

in which N_0 and N are respectively the initial and residual number of microorganisms, t the holding time at temperature θ and D_θ the decimal reduction time, that is to say the time required in order to reduce the microorganism population to 1/10 of the original value. This time is dependent on the microorganism and the product (pH, a_w) considered. For practical purposes, it is important to realize that complete destruction is never achieved and that the result is affected by initial contamination N_0. On the other hand, an increase in temperature results in a decrease of the D_θ value.

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The relationship between D_θ and temperature θ is given by:

$$\log \frac{D_\theta}{D_{\theta ref}} = \frac{\theta_{ref} - \theta}{z}$$

where $D_{\theta ref}$ is the value of D_θ at a reference temperature θ_{ref}, and z the increase in temperature necessary to increase the lethality of the heat treatment by a factor of 10. As an example, D_{53} and z are respectively equal to 12.6 minutes and 5.1°C for *S.* Enteritidis in liquid egg white (Denis et al., 1995). These equations makes it possible to define the equivalent time at a given temperature as compared to a reference treatment. The usual applied treatment is from 65 to 68°C held for 5 to 6 min for whole egg and yolk, and this enables satisfactory levels of microbial destruction. For liquid egg white, the pasteurization treatment consists of 57°C held for 2 to 5 min: from the previous values of D_θ and z, we can assume that this treatment results only in 1 to 2.5 log reduction of *S.* Enteritidis. This latter treatment is thus only partially effective, and leads to an increase in viscosity and turbidity, gelation and decrease in the whipping capacities due to protein denaturation. The thermal sensitiveness of these being lower at low a_w values, heat treatment of dried egg white is a way to achieve microorganism destruction. In France, two types of treatment are used: standard storage for about 15 days at 67°C and storage at 75°C-80°C for 15 days used to improve the functional properties of the dried egg white (whipping and gelling), which leads to high gel-type egg white. Recently Baron et al. (2003), investigated the effect of these dry heating treatments on the functional properties and subsequent ability of egg white to resist *Salmonella* growth after reconstitution. The storage at 67 or 75°C, even for a short time (one day), was very efficient in eliminating *Salmonella* and endogenous microflora. However, our results indicate a limitation to this treatment : the heating of dried egg white could affect, in a more or less significant way, the natural ability of this product to limit bacterial growth after reconstitution in water. This loss of bacteriostatic capacity, which could be attributed to the thermal denaturation of ovotransferin, is higher at 75°C than at 67°C. Nevertheless, the treatment at 75°C is used in industry particularly to amplify the functional properties of egg white.

In order to circumvent the difficulties of heat treatment, **non-thermal destruction processes**, including irradiation or pulse processes, are conceivable for egg product preservation.

Irradiation, realized by accelerated electrons or by gamma rays, is used by food industry to limit bacterial contamination. This treatment has been the subject of several studies since 1960. Irradiated products are satisfactory in many cases, but flavor changes can be expected. Gamma ray irradiation can be applied to frozen products, thereby eliminating the costs of thawing, heat pasteurization, and re-freezing. In France, the decree of October 10, 1990, authorizes the treatment of the liquid, dried or frozen egg white, with doses of 4 kGy. However, this process is often badly accepted by the consumers. Interest has recently increased for **pulse processes**, such as

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oscillating magnetic fields, intense light pulses and especially pulsed electric fields (PEF), since this represents one of the most promising technologies for the replacement of traditional thermal pasteurization. The PEF process consists of the application of short time high voltage pulses (2 μs to 1 ms) from 5 to 55 kV cm^{-1} to liquid food. Few studies concerned micro-organism inactivation in egg products by this process, which allowed however to prove its technical feasibility. Thus, the PEF process makes it possible to obtain 6 log reduction of *E. coli* at 37°C in whole egg (Martin-Belloso et al., 1997) and 3.5 log reduction of *S.* Enteritidis at 30°C in egg white, without apparent protein denaturation (Jeantet et al., 1999). This level of decontamination, higher than that obtained in liquid egg white pasteurization, remains, however, insufficient to guarantee the hygienic quality of industrial products. In addition, a recent study showed that the destruction of *Salmonella* is limited in the presence of egg white proteins. The destruction obtained previously would correspond consequently to a coupled PEF and heating effect (ohmic heating of the product during the treatment). Then, a solution could consist in combining different treatments.
Egg products are both a major source of microbial contamination and a complex raw material, which is difficult to treat, with regards to its viscosity and high shearing and heating sensitiveness of its proteins. Currently, refrigeration, freezing, sugar or salt addition and drying, are the only processes used on a mass production scale in France. These processes allow the microbial development to be limited more or less effectively. Concerning destruction processes, heat treatment is the only one used now, even if its effectiveness is not entirely satisfactory in the case of egg white. Non thermal processes can be considered, provided that they can compete the heat treatment in terms of microbial destruction, preservation of functional and organoleptic properties and cost, without forgetting perception by the consumer and regulation.

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Table 1. Composition and some properties of the major egg white proteins (Mine, 2002).

Protein	Albumen (% dry mass)	pI [1] (pH unit)	Mw [2] (kDa)	Td [3] (°C)	Main biological or technological functionality
Ovalbumin	54	4,5	44,5	84,0	Antigenic and allergenic properties; gelling effect
Ovotransferin	12 – 13	6,1	77,7	61,0	Binds iron and metallic ions (AE [4])
Ovomucoid	11	4,1	28,0	77,0	Inhibits serin protease (AE [4])
G2 Globulin	4	5,5	49,0	92,5	Foaming effect
G3 Globulin	4	5,8	49,0	-	Foaming effect
Ovomucin	3,5	4,5 – 5,0	$5,5 – 8,3.10^3$	-	Foam stabilizer at low temperature viscous effect
Lysozym	3,4	10,7	14,3	75,0	Lyses Gram+ bacteria (AE [4])
Ovo-inhibitor	1,5	-	49,0	-	Inhibits serin protease (AE [4])
Flavoprotein	0,8	-	32,0	-	Binds riboflavin (AE [4])
Ovostatin	0,5	-	$0,8 – 0,9.10^3$	-	Inhibits many protease (AE [4])
Avidin	0,05	10,0	68,3	-	Binds biotin (AE [4])

[1]: isoelectric point; [2]: molecular weight; [3]: denaturation temperature; [4]: antimicrobial effect

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GROWTH OF *SALMONELLA* SEROVARS IN HEN'S EGG ALBUMEN AS AFFECTED BY STORAGE

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Keywords: growth, *Salmonella*, egg albumen, storage

Abstract

Salmonella enterica serovars were tested for growth at 20°C in separated albumen. The albumen was either fresh or stored for up to 3 weeks prior to inoculation (p.i.). The serovar Enteritidis did not behave differently than the other serovars. A pronounced growth occurred more frequently and up to 1 log unit higher level in fresh albumen than in albumen stored p.i. This was at least partly explained by a pH effect. Growth was similar when the albumen had been stored p.i. in the absence or presence of yolk. Growth of *Salmonella* in the albumen of shell eggs also occurred more frequently in fresh eggs compared to eggs stored p.i. Thus cooling practices shortly after lay are recommended to prevent *Salmonella* from growing in eggs.

Résumé

La croissance de différents sérovars de Salmonella enterica a été testée à 20°C dans de l'albumen. L'albumen utilisé était d'une part frais, d'autre part stocké 3 semaines avant l'inoculation. Le sérovar Enteritidis a un comportement identique aux autres. L'albumen frais est un meilleur support (> 1 log) de croissance que l'albumen stocké, qui était en partie expliqué par un effet du PH. La croissance est la même que l'albumen ait été stocké en présence du jaune ou non. La croissance des salmonelles dans l'albumen des œufs en coquilles apparaît plus fréquemment dans les œufs frais que dans ceux qui sont stockés avant inoculation. Malgré tout, la réfrigération des œufs au plus tôt après la collecte est recommandée pour éviter la multiplication de salmonelles.

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Introduction

Salmonella enterica serovar Enteritidis is a common foodborne pathogen, the transmission of which is primarily associated with the consumption of contaminated shell eggs. There is no agreement about the growth of *Salmonella* when deposited in egg albumen near room temperature. A poor growth of *Salmonella* in fresh albumen near room temperature has been observed by some authors (e.g. Gast and Holt, 2001). Lysozyme, ovotransferrin (which deprives the microorganisms of iron) and the alkaline pH have been cited as the main defences against microbial growth in albumen (Mayes and Takeballi, 1983). According to Humphrey et al. (1991), growth will only occur when the egg's age > 21 days if held at 20°C. Upon that storage, nutrients or some factors negating the inhibitory properties of the albumen possibly leaked out from the yolk, because of alterations in the structure of the yolk membrane. In other studies (e.g. Schoeni et al., 1995), *Salmonella* was however able to grow in separated albumen from fresh eggs. Our study was conducted to aid solving this problem of conflicting results of growth/no growth of *Salmonella* in fresh and stored albumen.

Material and methods

Eggs. Intact eggs without faecal contamination from ISA-Brown Warren hens, collected at the day of lay, were used. The eggs were stored at 20°C until use.

Bacterial strains and cultures. Eleven *S.* Enteritidis strains and 5 other *S. enterica* serovars (2 Typhimurium, 1 Senftenberg, 1 Stanleyville, 1 Mbandaka, and 1 Blockley) were used. Overnight grown cultures were diluted with Ringer and used for inoculations.

Inoculation of *Salmonella* strains into separated albumen. Pooled albumen was divided into 60 ml portions that were inoculated with ± 39 cfu per ml albumen. *Salmonella* counts were determined after 6, 13 and 23 days of incubation at 20°C.

Growth of *Salmonella* strains in separated albumen from fresh eggs and in albumen separated from eggs stored prior to inoculation (p.i.) in the shell. Separated albumen samples taken from fresh and stored —at 20°C for 3 days and 1, 2 or 3 weeks— eggs were inoculated with one of the 17 strains.

Growth of *Salmonella* strains in separated albumen from fresh eggs and in albumen separated before storage. Pooled albumen of fresh eggs was divided in bottles, placed at 20°C and inoculated 2 and 3 weeks later with one of 4 strains of which 2 *S.* Enteritidis, 1 *S.* Typhimurium, and 1 *S.* Senftenberg strain.

Influence of pH on the growth of *S.* Enteritidis MB1409 in separated albumen from fresh eggs. The effect of the pH of albumen on the growth of *S.* Enteritidis

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MB1409 was assessed by comparing its growth in fresh albumen having either a natural pH (8.36) or an adjusted pH (9.31).

Growth of *Salmonella* strains in albumen within whole shell eggs. To investigate *Salmonella* growth in the albumen inside a whole shell egg, ± 8.2 cfu per egg were inoculated into the albumen (2 cm depth at the two o'clock position). Four strains of which 2 *S.* Enteritidis, 1 *S.* Typhimurium, and 1 *S.* Senftenberg strain were used. Both fresh eggs and eggs stored for 2 and 3 weeks p.i. at 20°C were used. After 6, 13 and 23 days of incubation at 20°C, the *Salmonella* levels were determined in yolk and albumen.

Enumeration of *Salmonella*. Enumeration of *Salmonella* was done by serial decimal dilution in Ringer, plating out on TSA agar and incubation overnight at 37°C. In accordance to Cogan et al. (2001), contamination levels > 10^4 cfu per ml separated albumen and > 10^6 cfu per ml yolk or albumen when whole shell eggs were inoculated, were assumed as generalised growth.

Results and Discussion

Growth of *Salmonella* strains in separated albumen from fresh eggs and in albumen separated from eggs stored p.i. in the shell. Growth rates of the 17 strains in separated albumen varied, also among repeats. No clear differences between strains could be observed. The number of samples showing generalised growth and the contamination levels were not significantly different when *S.* Enteritidis or other serovars were inoculated in albumen, but were significantly affected by the storage time p.i. and the incubation time (Fig. 1).

Hence, our results do not indicate that the serovar Enteritidis is better adapted to grow in albumen than other serovars, in contrast to what has been found by Lock and Board (1992). From a storage time of 2 weeks p.i. onwards, the ability of *Salmonella* to grow in separated albumen decreased significantly. This lower percentage of

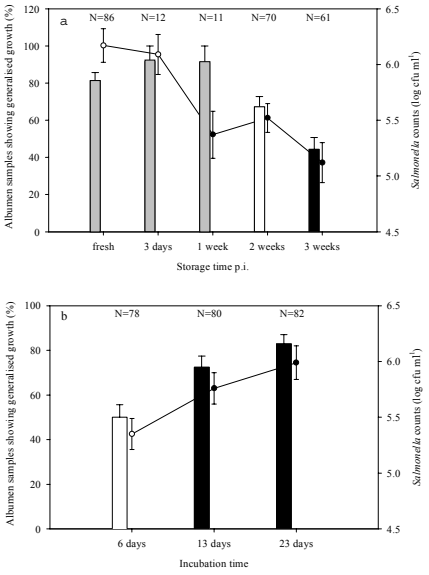

Fig. 1. Effect of storage time p.i. (a) and incubation time (b) on the growth of *Salmonella* in separated albumen at 20°C. Albumen was stored p.i. at 20°C in the whole shell egg. Values represent mean ± s.e. The number of samples (N) is given. Bars and symbols shaded differently are significantly different.

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generalised growth was accompanied by lower *Salmonella* counts. Incubating separated albumen samples for 13 and 23 days, led to a higher percentage of samples showing generalised growth and higher *Salmonella* counts compared to an incubation period of 6 days.

Growth of *Salmonella* strains in separated albumen from fresh eggs and in albumen separated before storage. To study the influence of the presence of yolk during the storage of albumen within the shell eggs p.i., the growth of 4 selected *Salmonella* strains has been simultaneously studied in albumen that had been stored separated from the yolk for 2 and 3 weeks p.i. Growth was not affected by the storage condition p.i. Hence, we concluded that there is no indication that leakage from the yolk takes place during the 3 weeks storage at 20°C.

Influence of pH on the growth of *S.* Enteritidis MB1409 in separated albumen from fresh eggs. It was assumed that the enhanced growth in fresh compared to stored albumen could have been caused by the lower pH in the former.

The effect of the pH-value of albumen on the growth of *S.* Enteritidis MB1409 was assessed by comparing its growth in fresh albumen at its natural pH with fresh albumen adjusted to a pH-value corresponding to 2-3 weeks stored albumen (Fig. 2). At its natural pH, generalised growth occurred in 93% of samples, at all incubation times. For the samples adjusted to the pH of stored albumen, 60% of samples showed generalised growth after 6 days incubation and 80% of samples after 13 and 23 days of incubation. Compared to samples with natural pH, *Salmonella* counts were about 1 log unit lower in the samples with adjusted pH. Thus, the influence of the pH itself or the influence of the pH on the albumen constituents may at least partly have led to a better growth in fresh albumen. Baron et al. (1997) have indicated that ovotransferrin, or iron deficiency resulting from iron binding to ovotransferrin, was the major protein or mechanism implicated in the inhibition of the growth of *S.* Enteritidis in albumen. At pH 9.0, iron is more tightly bound to ovotransferrin and cannot be removed by enterochelin (Tranter and Board, 1984). Hence, at the lower pH of fresh albumen more iron may be available for *Salmonella* to grow.

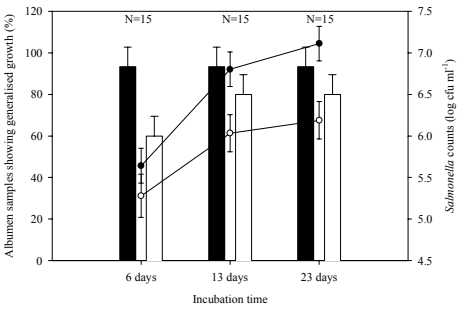

Fig. 2. Effect of pH of fresh albumen on the growth of *Salmonella* in separated albumen at 20°C as a function of the incubation time. The pH of fresh albumen was natural (filled bars and symbols) or adjusted to pH 9.31 (open bars and symbols). Values represent mean ± s.e. The number of samples (N) is given.

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Growth of *Salmonella* inoculated in albumen within whole shell eggs. When the albumen within the whole shell egg was inoculated with one of the strains *S.* Enteritidis MB1222, *S.* Enteritidis MB1409, *S.* Typhi-murium MB1142, or *S.* Senftenberg MB1559, at a level of ± 8.2 cfu per egg, the serovar and the incubation time did not significantly affect the number of eggs showing generalised growth and the *Salmonella* counts. The effect of storage of the eggs p.i. was highly significant ($P < 0.01$) as demonstrated in Table 1. Again, the best growth (percentage and count) was achieved when inoculating fresh albumen. About 50% of the fresh eggs allowed generalised growth compared to less than 10% at 2 and 3 weeks storage p.i. The *Salmonella* counts in the albumen were similar as those in the yolk, and were about 1 log unit higher in case of fresh eggs compared to the ones stored p.i. It can be concluded from our study that cooling practices are recommended shortly after lay to prevent *Salmonella* from growing in eggs, even when the albumen gets contaminated.

Table 1. Effect of the storage time p.i. on *Salmonella* growth in whole shell eggs at 20°C. The albumen within the whole egg was inoculated with ± 8.2 cfu.

	Storage time p.i.		
	Fresh	2 weeks	3 weeks
Number of replications	24	22	23
Eggs showing generalised growth (%)[a]	50.0 ± 7A	0 ± 7B	8.7 ± 7B
Counts in albumen (log cfu ml^{-1})[a]	8.50 ± 0.2A	-[b]	7.08 ± 0.4B
Counts in yolk (log cfu ml^{-1})[a]	8.68 ± 0.08A	-[b]	7.84 ± 0.2B

Values are means ± s.e.
[a]Values in each row bearing different uppercase letters do not differ at $P = 0.05$.
[b]The mean is not given because generalised growth was not observed.

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Acknowledgements

The authors would like to thank the financial support from the European Community, project QLK5-CT-2001-01606 and from the Belgian Ministry of Small Enterprises, Traders and Agriculture, Directorate Research and Development, project S-5912.

CONTENTS

POTENTIATION OF *SALMONELLA* ENTERITIDIS GROWTH IN SHELL EGGS

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Keywords: Eggs, *Salmonella* enteritidis, vitelline membrane, temperature

Abstract

Infected hens can transovarianly transmit *Salmonella* Enteritidis (SE), and produce eggs that are contaminated at time of lay. SE cells are deposited in the albumen in close proximity to the vitelline membrane. While constituents of the albumen inhibit SE growth, introduction of minute amounts of yolk into albumen allows rapid SE growth. We have also demonstrated SE growth in albumen of inoculated eggs subjected to temperatures representative of commercial holding after packing. In contrast, SE does not grow in albumen of inoculated eggs rapidly cooled to 7 C. The vitelline membrane, whose integrity is time-temperature dependent, appears to be the key factor affecting SE growth in shell eggs. Growth models developed in the UK have related SE growth to loss of vitelline membrane integrity. Further research was conducted to more clearly define time-temperature relationships needed to permit SE growth in albumen, and to directly correlate SE growth to vitelline membrane strength. Growth patterns were similar, but times before significant SE growth were greater as compared to the UK models. Force required to break vitelline membranes linearly declined over time, and rates were temperature-dependent. Membrane breaking strength of $\leq$1.5 g seems to correlate with rapid SE growth.

Résumé

Les œufs infectés par Salmonella Enteritidis (SE) peuvent produire des œufs contaminés au moment de la ponte. Les SE sont déposées dans l'albumen, dans l'environnement proche de la membrane vitelline. Alors que les constituants de

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l'albumen inhibent la croissance de SE, l'introduction de quantités très faibles de jaune permet une croissance rapide de SE. La croissance de SE dans l'albumen aux températures habituelles de stockage a été démontrée. Au contraire, SE ne croît pas dans l'albumen des œufs rapidement réfrigérés à 7 °C. L'intégrité de la membrane vitelline, qui dépend du temps et de la température, est l'un des facteurs clé qui affecte la croissance de SE. Les modèles de croissance développés au Royaume Uni ont démontré un lien entre la croissance de SE et la perte d'intégrité de la membrane vitelline. Des recherches ont été mises en œuvre, afin d'établir les conditions de temps/température nécessaires à la croissance de SE dans l'albumen et le lien éventuel avec la solidité de la membrane vitelline. Les taux de croissance sont similaires à ceux des modèles anglais, mais les temps de latence sont plus longs que ceux proposés par ces mêmes modèles. La résistance de la membrane vitelline diminue avec le temps et la température. Une force de rupture de la membrane ≤ 1,5 kg semble corréler avec une croissance rapide de SE.

Introduction

Salmonellosis continues to be the most commonly reported foodborne disease in developed countries, and *Salmonella enterica* serotype Enteritidis (SE) is among the most frequently isolated serotypes (CDC, 2000). The emergence of SE infections in humans, which began to be noted by public health officials in the mid to late 1980's, has been attributed to contaminated table (shell) eggs (St. Louis *et al.,* 1988; CDC, 2000). Table eggs can be contaminated with SE via a transovarian route before oviposition, meaning that the egg is contaminated during its formation by the hen, before the shell is added. SE appears to be deposited in the albumen or on the vitelline membrane (Gast and Beard, 1990; Humphrey, *et al*., 1991). However, SE contamination of eggs is rare. The USDA estimates a 1 in 20,000 egg contamination rate. Furthermore, contaminated eggs typically contain <100 cells/egg at time of lay (Humphrey, 1994).

Although SE contamination of eggs occurs at a low rate even in positive flocks and only low levels of SE are found in positive eggs, there is concern that SE can grow if eggs are improperly handled (Bradshaw, *et al.*, 1990). However, eggs contain several antimicrobial agents which reduce the chances of bacteria surviving and multiplying, thereby serving to maintain microbial stability. After oviposition there are both chemical and physical changes in the egg which cause losses of quality, which appear to affect the egg's antimicrobial properties. Time, temperature, humidity and handling are factors associated with quality loss in eggs (Stadleman, 1995). One key change is a decrease in strength of the vitelline membrane from the time of lay. This decrease in strength is due to loss of carbon dioxide into the environment and movement of water

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from albumen into the yolk. (Romanoff and Romanoff, 1949). Also, iron over time will move from the yolk into the albumen (Schaible *et al.*, 1944). These chemical and physical changes within eggs, may, in part, account for results of important research on the growth of SE in eggs. Growth of SE in albumen close to yolk was affected by storage time/temperature (Humphrey, 1993; 1994), suggesting that the yolk membrane degrades over time, allowing yolk components to migrate into the albumen and/or SE to gain access to the yolk. Presumptively, either of these situations would create an environment in which SE could grow rapidly if permissive temperatures exist.

Further research has been conducted to determine factors that affect ability of SE to grow in albumen. The primary objective was to more clearly define time-temperature relationships needed to permit SE growth in albumen, and to directly correlate SE growth to vitelline membrane strength.

Material and Methods

SE growth in albumen. Eggs were obtained within two hours of lay, dipped into absolute ethanol and allowed to air dry to disinfect the shell. Eggs were aseptically cracked, and yolk and albumen were separated using a sterile egg separator. Contents were placed in sterile beakers, and mixed using a sterile spatula. After all yolks and albumen had been mixed, appropriate amounts were pipetted into sterile flasks to give 100 ml of the following mixtures (vol:vol): 100:0, 50:50,30:70, 10:90, 5:95, 1:99 and 0:100 yolk to albumen. A sterile foam stopper was placed in each flask and covered with aluminum foil. Each flask was inoculated (100 cfu/ml) with SE or left uninoculated (control). Flasks were stored at 18C for 7 d post inoculation, and SE populations were enumerated daily.

Cryogenic Rapid Cooling of Eggs. Eggs were obtained within 12 h of lay. A composite suspension of SE strains Benson, Puerto Rico, and Rochester was injected into eggs. This inoculum (50 *u*l) was deposited at center albumen next to yolk to at ~10^7 (high inoculum) or ~10^2 cfu/egg (low inoculum). After inoculation, the shell was sealed using a droplet of Duro^{TM} Super Glue (Loctite Corp., Cleveland, OH, USA). After the glue had dried, eggs were subjected to two cooling treatments : traditional vs rapid cryogenic. Egg cooling data collected from actual table egg processing plants (Anderson *et al.,* 1992) were used to establish traditional cooling curves. Traditional cooling was achieved by placing eggs into the low temperature incubator in which the temperature was 25.5 C on day 1, 18.3 C on day 2, 12.8 C on day 3, 11.1 C on day 4, and 7.0 C where the temperature remained until the end of the study. Eggs rapidly cooled cryogenically were placed into traditional cardboard flats (2.5 dozen) before being placed into the cryogenic cooler provided by Praxair (Burr Ridge, IL). Cryogenically cooled eggs were cooled to approximately 7 C in about 6 min with

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gaseous CO_2, then placed in cartons before placement in a low temperature incubator at 7 C for 15 d. On prescribed days of storage, five eggs from each treatment group were taken to enumerate SE populations in internal contents.

Time-Temperature Effects on SE Growth and Vitelline Membrane Strength. Grade A shell eggs were obtained from a commercial producer, then stored at four different temperatures. Periodically, subsets of eggs from each temperature group were inoculated with SE, and changes in populations of SE were determined using the procedure of Humphrey (1993, 1994). At key sample times, additional eggs were analyzed to determine vitelline membrane strength. Compression measurements were made using the TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) with a 5 Kg load cell and a 0.1 gram trigger setting. Pressure was applied at a rate of 3.2 mm/sec with the instrument set at ten grams full scale. A 1 mm, rounded end, stainless steel probe was used to apply direct pressure to the yolk vitelline membrane until membrane rupture occurred. Membrane breaking strength was reported as grams force require for rupture.

Results and Discussion

SE growth in albumen. Generally, as percentage of yolk increased in albumen, SE growth increased. At 6 d, SE populations were $\geq$9 $\log_{10}$ cfu/ml in 100:0 and 50:50 yolk:albumen mixtures, and growth curves were identical (data not shown). In contrast, no growth occurred when SE was placed in 0:100 yolk:albumen mixture (Fig 1). At the other yolk:albumen ratios, response varied (Fig 1). In 30:70 yolk:albumen SE reached $\geq$9 $\log_{10}$ cfu/ml by 5 d. In 10:90 and 5 :95 yolk:albumen, SE increased gradually, reaching 8 and 7 $\log_{10}$ cfu/ml, respectively by day 6. In 1:99 yolk:albumen SE populations increased to 2.01 $\log_{10}$ cfu/ml at day 6. Again, no growth occurred in the 0:100 yolk:albumen mixture. These data, indicate that as yolk is introduced into albumen, antimicrobial effects are lost and SE can grow in albumen.

Cryogenic Rapid Cooling of Eggs. At the high inoculum level (~10^7 cfu/egg), eggs cooled traditionally had a SE population of 7.12 $\log_{10}$ cfu/ml after one day of storage, which increased gradually to 7.90 $\log_{10}$ cfu/ml on day 15. Eggs rapidly cooled cryogenically exhibited a gradual decrease in the SE population over time. At 1 d, SE population was 7.02 $\log_{10}$ cfu/ml, which decreased to 6.26 $\log_{10}$ cfu/ml by day 15 (Fig 2). At the low inoculum levels (~160 cfu/egg), the SE population in cryogenically cooled eggs remained at or near the initial level throughout storage, whereas SE grew in albumen of eggs subjected to the traditional cooling treatment (Fig 2). Extended holding times at temperatures representative of the traditional cooling treatment appear to create an environment in the albmunen that is permissive to SE growth, wherease rapid cooling and holding at 7C effectively prevents SE growth.

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Time-Temperature Effects on SE Growth and Vitelline Membrane Strength. Both SE growth and membrane deterioration were affected by the temperature at which the eggs were stored. As hypothesized, the ability of SE to grow in albumen corresponded to a decline in the force required to break the vitelline membrane. Only a small percentage of eggs that were stored at 4 or 10 C permitted SE proliferation, indicating that membrane integrity was maintained (Fig 3). The force required to break the vitelline membrane of eggs stored at these two temperatures substantiate that membrane integrity was maintained. However, it should be noted that membrane strength declined over time, and the rate of deterioration was greater at 10 C than at 4 C. In eggs stored at 10 C, the force required to break the vitelline membrane declined from 2.33 g to 1.56 at 8 weeks of storage. At 9 weeks, 10 % of the eggs permitted significant SE growth(>3 log increase), the highest percentage noted at this temperature. At 20 C, there was a rapid deterioration of vitelline membrane strength, and significant SE growth was noted at 3 weeks of storage (Fig 3). By 5 weeks of storage, 82% of eggs permitted a > 3 log increase in SE growth. At 30 C (Fig 4), a rapid decline in membrane breaking force was observed. This force declined from 2.33 g to 1.4 g with 12 days of storage, and the membrane continued to loose integrity throughout the entire storage period. At 12 days of storage, 58 % of the eggs permitted > 3 log increase in SE growth (Fig 4). A membrane breaking strength of ~1.5 g (as measured with the technique applied in the present study) appeared to be the point at which vitelline membrane integrity is compromised to a point that SE growth is permissible in the albumen.

Conclusion

While the general assumption that that egg albumen will not support growth of SE is true, chemical and physical changes within the egg can compromise this antimicrobial effect to the point SE can grow. Segration of yolk and albumen constitiuents is a major factor affecting SE growth in albumen. Conditions, including temperature abuse, can potentiate SE growth; therefore, eggs should be rapidly cooled to and handled at temperatures to maintain chemical and physical integrety of vitelline membrane and possibly other egg components.

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Acknowledgement

The authors acknowledge the assistance of LaVonda Hughes, Joshua Gurtler and Joanna Tharrington for their excellent technical assistance.

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Figures

Figure 1. Population of SE in various mixtures of yolk:albumen after incubation at 18 C for 6 days.

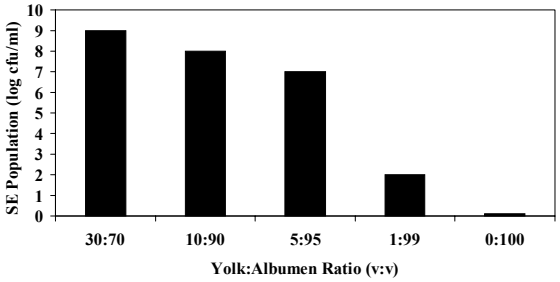

Figure 2. Effects of traditional vs rapid cryogenic cooling on low and high levels of SE in albumen of shell eggs.

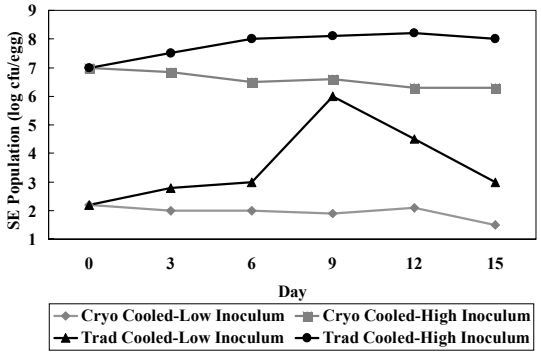

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Figure 3. Effect of storage time and temperature on vitelline membrane strength (lines) and on ability of SE to grow in albumen (bars).

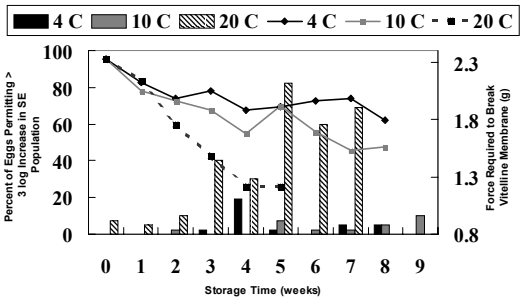
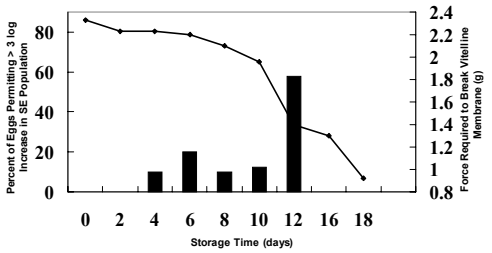


Figure 4. Effect of storage time at 30 C temperature on vitelline membrane strength (lines) and on ability of *Salmonella* Enteritidis to grow in albumen (bars).



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YEAST GLUCOMANNANS PREVENT DETERIORATION OF QUAIL EGG QUALITY DURING AUROFUSARINOTOXICOSIS

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Keywords**:** Aurofusarin; Quail; Mycosorb; vitamin E; polyunsaturated fatty acids

Abstract

The aim of this study was to evaluate effects of modified glucomannans (Mycosorb^{TM}) on egg yolk and liver of the day-old quail after aurofusarin inclusion in the maternal diet. Fifty four 45-day-old Japanese quail were divided into three groups and were fed ad libitum a corn-soya diet balanced in all nutrients. The diet of the experimental quails was supplemented with aurofusarin at the level of 26.4 mg/kg feed in the form of *Fusarium graminearum* culture enriched with aurofusarin or with aurofusarin plus Mycosorb^{TM} at 1 g/kg feed. Inclusion of aurofusarin in the maternal diet was associated with decreased proportions of docosahexaenoic acid (DHA) and increased proportions of linoleic acid (LA) in major lipid fractions of the egg yolk as well as with decreased concentrations of α- and γ-tocopherols, α- and γ-tocotrienols in egg yolk and liver of a day-old quail. Inclusion of modified glucomannans (Mycosorb^{TM}) into the quail diet simultaneously with aurofusarin showed a significant protective effect against changes in PUFA and antioxidant composition in the egg yolk and liver of quail.

Resume

Cette étude consiste à évaluer les effets de l'administration de glucomannanes modifiés (mycosorbTM) sur le jaune d'œuf et le foie de la caille de un jour après adjonction d'aurofusarine dans le régime maternel. 54 cailles de un jour ont été divisées en trois groupes et nourries ad libitum avec un aliment soja-blé équilibré. Les lots expérimentaux ont été supplémentés avec d'une part, de l'aurofusarine à 26,4 mg/kg d'aliment maternel, sous la forme d'une culture de fusarium graminearum enrichie en aurofusarium; et d'autre part, du même régime additionné de

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MycosorbTM : 1g/kg d'aliment maternel. L'ajout d'aurofusarine dans le régime maternel a entraîné une baisse de l'acide docosahexaenoique (DHA) et une augmentation de l'acide linoléique (LA) dans la fraction lipidique majeure du jaune d'œuf ainsi qu'une diminution des α et γ tocophérols, et α et γ tocotriénols dans le vitellus et le foie des cailles de un jour. L'adjonction de MycosorbTM dans le régime alimentaire maternel contrebalance ces effets négatifs sur la composition en PUFA et antioxydants du jaune d'œuf et du foi.

Introduction

Mycotoxins are toxic secondary metabolites of moulds and their negative effects on poultry production are incalculable. In general mycotoxins are considered to be unavoidable contaminants in foods and feeds and are a major problem all over the world (Wood, 1992). More than 300 mycotoxins have been shown to induce signs of toxicity in mammalian and avian species (Fink-Gremmels, 1999; Leeson *et al.*, 1995) and this number is increasing. It has been estimated that 25% of the world's cereal grains are contaminated with mycotoxins (Fink-Gremmels, 1999). Apart from acute and chronic toxic effects on animal and human health called mycotoxicosis, some mycotoxins are proved or suspected human carcinogens (Peraica and Domijan, 2001; Peraica et al., 1999).

Among all mycotoxins, those from *Fusarium* species are considered to be important contaminants of poultry feed (Smith, 1990, 1992; Leeson et al., 1995; Kotyk, 1999). Trichothecenes, zearalenone, fumonisins, moniliformin and fusaric acid are the major *Fusarium* mycotoxins occurring on a worldwide basis in cereal grains, animal feeds and forages (D'Mello *et al.*, 1999; Hussein and Brasel, 2001). Furthermore, the trichothecene mycotoxins themselves comprise a vast group of over 100 fungal compounds with the same basic structure (Leeson *et al.*, 1995). In many cases these mycotoxins can be found in combination in contaminated feed with the additive toxic effects (Raju and Devegowda, 2000; Swamy, 2002; 2002a). Fusarium fungi also produce some pigments (Duran et al., 2002; Medentsev and Akimenko, 1998; Parisot et al., 1990), which until recently have been ignored. One of such pigment, aurofusarin, a dimeric naphthoquinone, was recently identified as a contaminant of grain in Ukraine (Kotyk, 1999). In particular this mycotoxin has been shown to have a profound effect on egg quality (Kotyk et al., 1990; 1995; Dvorska 2001). However, there were no clinical signs of toxicosis. On the other hand, aurofusarin compromised the immune system of laying chickens (Sakhatsky, 1999) and quails (Dvorska et al., 2001) and decreased fertility and hatchability. On the biochemical level, aurofusarin was responsible for significant changes in fatty acid and antioxidant profiles of quail eggs (Dvorska et al., 2001a). Furthermore, the antioxidant system of the developing

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quail embryo was shown to be compromised as a result of aurofusarin inclusion in the maternal diet (Dvorska et al., 2001).
Our previous study (Dvorska and Surai, 2002) showed a protective effect of modified glucomannans against T-2 toxicosis in quail. In particular, protective effects of the glucomannans were associated with prevention of antioxidant depletion in quail tissues due to T-2 toxicosis. The aim of the present work was to study effects of modified glucomannans (Mycosorb^{TM}) on egg yolk and liver of the day-old quail after aurofusarin inclusion into the maternal diet.

Materials and Methods

Fifty four 45-day-old Japanese quails (*Coturnix Japonica*, egg-type reared in Ukraine) were divided into three groups (control and two experimental groups, 15 females + 3 males in each group) and were fed ad libitum on a corn-soya diet balanced in all nutrients. The diet of the experimental quails was supplemented with aurofusarin at the level of 26.4 mg/kg feed in the form of *Fusarium graminearum* culture enriched with aurofusarin or with aurofusarin plus Mycosorb^{TM} (a trademark of Alltech, Inc. that has been registered with the U.S. Trademark Office, 1 g/kg feed). The mycotoxin dose was tested by Kotyk (1999) and Sakhatsky (1999) and shown to have a pronounced negative effect on egg quality and immunity of laying hens as well as altering the fatty acid and antioxidant profile of quail eggs and tissues (Dvorska et al., 2001a; 2002). The culture was a gift from A.N. Kotyk (Poultry Research Institute, Borky, Ukraine) and was produced by growing *Fusarium graminearum* B-5 on millet. The millet was steamed, dried and ground to a fine powder and analyzed for aurofusarin content by a bioautographic method (Kotyk and Trufanova, 1998). It was confirmed that the culture contained exclusively aurofusarin. Spectral analyses of aurofusarin revealed three major peaks at 248, 265 and 345 nm, which are characteristic for aurofusarin (Kotyk, 1999). Eggs obtained after 8 weeks of feeding were analysed and incubated in standard conditions of 37.5^{o}C/55%RH. Tissue samples were harvested from day-old quails. 37.5^{o}C/55%RH. Tissue samples were harvested from day-old quails.
Tocopherol and tocotrienol extraction from egg yolk and tissues was performed as previously described (Dvorska et al., 2002) and was determined using an HPLC system. Lipids analysis were perfermed by gas-liquid chromatography (Christie, 1982). Results are presented as mean (± S.E.) of measurements on 6 egg yolks or 5 livers from each group. Statistical analysis was performed by Student's t-test.

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Results and Discussion

Aurofusarin consumption was associated with significant changes in the fatty acid profile of egg yolk. The most dramatic changes were observed in the phospholipid fraction. The proportion of linoleic acid (LA, 18:2n-6) increased by 35.6% ($P<0.05$) with a concomitant decrease in the proportion of docosahexaenoic acid (DHA, 22:6n-3) of 44.6% ($P<0.01$). At the same time, arachidonic acid proportion in the egg yolk lipids remained constant. Inclusion of dietary Mycosorb^{TM} prevented changes in the egg yolk fatty acid profile due to aurofusarin consumption. Changes in the LA proportion of the triacylglycerol fraction of the quail yolk were similar to those in phospholipid fraction with a significant ($P<0.01$) increase (by 43%) of this fatty acid proportion due to aurofusarin consumption. Mycosorb^{TM} incorporation into the diet prevented those changes in LA proportion in the egg yolk. In the cholesterol ester fraction the proportion of LA did not change, however, there was a significant ($P<0.01$) reduction in DHA proportion (by 49%) in this fraction as a result of aurofusarin presence in the diet. Mycosorb^{TM} inclusion into the quail diet decreased changes in DHA proportion in the cholesterol ester fraction; however, this preparation was not able to completely eliminate those changes. In the free fatty acid fraction there were also changes in PUFA profile as a result of aurofusarin inclusion in the maternal diet: LA and arachidonic acid proportions were increased by 65% ($P<0.01$) and 50% ($P<0.05$), respectively. At the same time DHA proportion in this lipid fraction decreased by 42% ($P<0.01$; Table 4). Mycosorb^{TM} inclusion in the diet helped to overcome changes in PUFA profile in the free fatty acid fraction of the egg yolk.

Aurofusarin consumption was associated with a significant reduction in concentration of all vitamin E forms studied (by 18% for tocopherols and 33-44% for tocotrienols) in the egg yolk ($P<0.05$; Table 5). At the same time a protective effect of Mycosorb^{TM} was indicated by return of vitamin E concentration to the control levels. Antioxidant system of the newly hatched quails was also compromised as a result of decreased vitamin E concentrations in the egg yolk (Table 6). For example, α- and γ-tocopherol concentrations in the liver of day-old quail decreased by 36% ($P<0.01$) and γ-tocotrienol by 35% ($P<0.05$). Mycosorb^{TM} consumption together with aurofusarin prevented not only changes in vitamin E concentration in the egg yolk, but also was protective in terms of maintaining antioxidant system activity of the newly hatched quail. In fact, vitamin E concentration in the liver of the 3 day-old quail group was not dissimilar to that of control birds.

Among hundreds of mycotoxins studied, aurofusarin has received only limited attention. However, from the data accumulated so far it is clear that this Fusarium pigment should be considered a mycotoxin. It has also been suggested that aurofusarin be considered a new crop pollutant (Dvorska et al., 2000). Grains contaminated with aurofusarin have a pinkish colour; and this pigment was detected in 11 samples of

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wheat growing in Ukraine in 1988-1990 (Kotyk and Trufanova, 1990). Indeed, one of the primary effects related to presence of this pigment is a change in egg yolk colour from yellow-orange to dark-brown with a greenish shade (Kotyk et al., 1990; Kotyk et al., 1995; Kotyk, 1999; Dvorska, 2001). Our previous study (Dvorska, 2001a) confirmed that aurofusarin is transferred from the feed to the egg yolk, and due to its own colour being pH-dependent could cause colour change from yellow via red and pink to violet. Therefore a combination of this colour and the carotenoid-based yellow colour of the yolk could be responsible for those changes. Furthermore, aurofusarin consumption is associated with deterioration of chicken meat quality (Dvorska, 2000; 2000a; 2000b), compromised immunity in of laying hens (Sakhatsky, 1999) and quails (Dvorska, 2001a; Dvorska et al., 2001) and decreased fertility and hatchability in chickens (Sakhatsky, 1999) and quail (Dvorska, 2001a; Dvorska et al., 2001).

This study confirmed our previous observations related to the profound effect of aurofusarin consumption on fatty acid composition of quail egg yolk (Dvorska et al., 2001a). Since similar diets were used, proportions of PUFAs in the egg yolk were similar to those in the previous study. The mechanisms of such action of aurofusarin are not clear at present, but the possibility of its involvement in the regulation of desaturase activity, similar to the tocopherol quinone (Infante, 1999) needs further investigation. In particular, a decreased vitamin E concentration in the egg yolk and probably in the quail liver could decrease synthesis of α-tocopherol quinone, which could affect DHA synthesis. On the other hand, because of the quinone structure of aurofusarin, it can directly interfere with α-tocopherol quinone action on fatty acid desaturases. In both cases, the decreased synthesis of DHA would be expected. There is also a possibility that aurofusarin could affect incorporation of individual fatty acids into newly synthesised VLDL in the liver of the hen. Indeed molecular mechanisms of the aurofusarin effect on lipid metabolism need further investigation.

Interference of aurofusarin with vitamin E accumulation in the egg yolk and its delivery to the developing embryo was revealed in this study. It seems likely that aurofusarin not only decreases vitamin E concentration in the egg yolk, but also affects its transfer to the developing embryo, since the difference between control and experimental groups became greater in the liver of the chick in comparison to the egg yolk. A compromised antioxidant system could be responsible for increased quail mortality at late stages of incubation due to aurofusarinotoxicosis (Dvorska, 2001). Pro-oxidant properties of many mycotoxins have received substantial attention in recent years (for review see Atroshi et al., 2002; Surai, 2002; Surai et al., 2002). Biochemical changes in mycotoxicosis are widespread and lipid peroxidation is regarded as one of the most important consequences of mycotoxicosis (Mezes et al., 1999). Aflatoxins, T-2 toxin, ochratoxins, fumonisins, DON and zearalenone possess pro-oxidant properties and are able to stimulate lipid peroxidation. Therefore this list should be extended to include aurofusarin. However, molecular mechanisms of lipid

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peroxidation stimulation by individual mycotoxins and their mixtures require further research.

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GENETIC RELATIONSHIPS BETWEEN *CAMPYLOBACTER COLI* ISOLATED FROM POULTRY

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Keywords: *Campylobacter coli*, phenotyping, genotyping, clonal relationships

Abstract

The aim of the study was to investigate the genetic relationships between twenty nine Campylobacter isolates collected from the same group of broilers at the abattoir. Each isolate was purified, then identified by using biochemical tests and multiplex PCR. Moreover, it was submitted to phenotypic characterisation by antibiotic resistance profile and biotyping, as well as to *Pst*I and *Hae*III ribotyping. All isolates were identified as *Campylobacter coli.* The phenotypic and *Pst*I ribotyping results were the same for all strains. However, *Hae*III was able to classified the isolates in five different groups. The results of this study show that a powerful genotyping method can be useful to differentiate isolates that, according to their phenotypic characteristics, might be erroneusly classified as clonal.

Résumé

L'objectif de cette étude était d'étudier les relations génétiques entre 29 isolats de Campylobacter recueillis parmi le même groupe de poulets à l'abattoir. Chaque isolat a été purifié et identifié par l'utilisation de tests biochimiques et de PCR multiple.
De plus il a été caractérisé au niveau phénotypique par le profil d'antibiotique résistance, le biotype et le génotype en utilisant les enzymes de restriction *Pst*I et *Hae*III. Tous les isolats ont été identifiés comme *Campylobacter coli.* Les résultats phénotypiques et de génotypique avec *Pst*I étaient les mêmes pour chaque isolat. Toutefois *Hae*III a été capable de classifier les isolats en cinq différents groupes. Les résultats de cette étude démontrent qu'une méthode génotypique peut être utile pour différencier les isolats, qui, à partir d'une seule caractérisation phénotypique, peuvent être classés dans le même clone par erreur.

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Introduction

Campylobacter spp. are among the most frequently reported causes of bacterial enteritis in developed countries. The majority of clinical reports from human cases is sporadic and outbreaks are rare (Frost, 2001). In industrialised countries, cases of human campylobacteriosis due to *C. jejuni* and *C. coli* are about 85% and 7%, respectively. *C. jejuni* and *C. coli* can be identified by using biochemical testes, and in particular the hippurate hydrolysis test (Barrett et al., 1988), or by using PCR (On et al., 2003). Denis *et al*. (1999) described a multiplex PCR (mPCR) method for the simultaneous detection of the *Campylobacter* genus, *C. jejuni* and *C. coli* by specific detection of 16S rRNA gene, *mapA* gene and *ceuE* gene, respectively. Once identified the Campylobacter isolates can be characterised by phenotypic methods such as biotyping and antibiotic resistance profile, as well as by genotipic methods The more commonly used genotyping techniques include pulsed field gel electrophoresis, randomly amplified polymorphic DNA analysis, flagellin gene restriction fragment length polymorphism analysis, restriction fragment length polymorphism analysis, multilocus sequence typing, and amplified fragment length polymorphism analysis (Wassenaar and Newell, 2000). However, only an automated technique, such as that represented by the automated ribotyping, may allow laboratories to apply molecular typing more broadly, due to its facility of use and speed of analysis. Moreover, the ribotypes collected all over the world can be easily shared between different laboratories using on line databases such as the PathogenTracker (www.pathogentracker.net). The RiboPrinter®, based on the principle of ribotyping, automates and standardises all process steps required, from cell lysis to image analysis, providing results within 8 h (Bruce, 1996). The Campylobacter ribotyping analysis can be performed by using *Pst*I or *Hae*III.
The aim of this study was to identify and characterise both by phenotypic and genotypic methods twenty nine Campylobacter isolates collected from the same flock of broilers to verify their genetic relationships.

Material and Methods

Sampling and isolation procedure
Seventy five cloacal swabs were obtained from single randomly selected broilers belonging to the same flock, at the abattoir. Each swab was transferred in tubes containing 10 ml of Preston broth (Oxoid), incubated at 42°C for 24 hours in microaerophilic atmosphere obtained using CampyGen (Oxoid). Following incubation, one loopful of culture was streaked onto mCCDA (Oxoid), incubated in modified

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atmosphere at 42°C for 48 hours. *Campylobacter* suspected colonies were subcultivated until monocultures were obtained. Curved or spiral Gram negative rods, catalase and oxidase positive were presumptively identified as *Campylobacter*. Twenty nine strains were randomly selected to be identified and typed.

Biochemical identification and biotyping

Indoxyl-acetate hydrolysis, rapid H_2S in FBP, hippurate hydrolysis test, DNAase test were performed according to the scheme described by Lior (1982).

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was performed by using the agar disk diffusion method using Mueller-Hinton agar supplemented with 5% sheep blood. The plates were incubated at 37°C for 24 hours in microaerophilic atmosphere. The following antibiotics were tested: enrofloxacin 5µg (ENR), norfloxacin 10µg (NOR) ciprofloxacin 5µg (CIP), nalidixic acid 30 µg (NA), tetracycline 30 µg (TE), erythromycin 15 µg (E), azithromycin 15 µg (AZM), gentamicin 10 µg (GM), streptomycin 10 µg (S), sulphamethoxazole + trimethoprim 25µg (SXT), cephalothin 30 µg (KF), chloramphenicol 10µg (CH). Interpretative criteria were in accordance with the NCCLS (1999) recommendations.

Multiplex PCR identification

The DNA to be used as target in the mPCR was extracted as described by Jackson et al. (1993). The mPCR was performed according to the protocol published by Manfreda et al. (2003). The expected PCR amplicons were at 857, 589 and 462 bp corresponding to the genus Campylobacter, to the species *C. jejuni* and to the species *C. coli*, respectively.

Automated ribotyping

Ribotyping was performed using the RiboPrinter® according to the manufacturer's instruction's (Bruce, 1996). *Pst*I and *Hae*III were used in different runs. The ribotype pattern for each isolate was automatically compared to patterns stored in the RiboPrinter® database. The profiles collected in this research were labelled with an alphanumeric code (i.e. 24 S3), that defined the ribotype of the isolate.

Results and discussion

Seventy four out of seventy five swabs were positive for *Campylobacter* spp. Based on biochemical tests, the 29 isolates selected for this study were identified as *C. coli*, biotype 1. The identification was confirmed also by using the mPCR. All isolates showed the same pattern of resistance to all the antimicrobials tested, except for gentamicin and chloramphenicol (Table1).

The same *Pst*I ribotyping profile, corresponding to the ribogroup 24 S3 (Figure 1) characterised all strains examined. However by using *Hae*III the isolates were

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clustered into five different ribogroups containing from one to thirteen strains (Table 2). The ribotyping profiles corresponding to these different ribogroups (Figure 2) were characterised by a similarity range from 82 to 46%. According to CAMPYNET, an EU–funded Network for coordination of molecular typing methods for *Campylobacter* (www.svs.dk/CampyNet), the definition of the ribotyping profiles obtained using *Pst*I can sometimes be problematic and may require visual assessment, whereas *Hae*III normally produces well separated bands (Fitzgerald et al., 1996). In this research that both *Pst*I and *Hae*III were able to produce ribotyping profiles characterised by well defined non overlapping bands. However *Pst*I was not able to discriminate any *C. coli* isolate whereas *HaeIII* showed a preatty high discriminatory power (SID=0.69). These results show that the use of a powerful genotyping method, such as that represent by *Hae*III ribotyping, can allow to discriminate isolates that according to phenotypic and other genotypic tests might seems clonal. The possibility to characterise the different strains that can infect broilers is fundamental to tracing the Campylobacter infection routes between animals, foods, and humans.

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Table 1- Antibiotic resistance profiles

Label	ENR	NOR	CIP	NA	TE	E	AZM	GM	S	SXT	KF	CH
BrIZS 1	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 2	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 3	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 4	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 5	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 6	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 7	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 8	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 9	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 10	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 11	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 12	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 13	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 14	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 16	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 17	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 18	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 19	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 20	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 21	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 22	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 23	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 24	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 25	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 26	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 27	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 28	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 29	R	R	R	R	R	R	R	S	R	R	R	S
BrIZS 30	R	R	R	R	R	R	R	S	R	R	R	S

Table 2 – *Hae*III ribotyping results

Ribogroup	N. isolates
150 S1	3
150 S3	2
150 S6	13
165 S2	10
168 S8	1

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Figure 1 – *Pst*I ribotyping profile corresponding to the ribogroup 24 S3

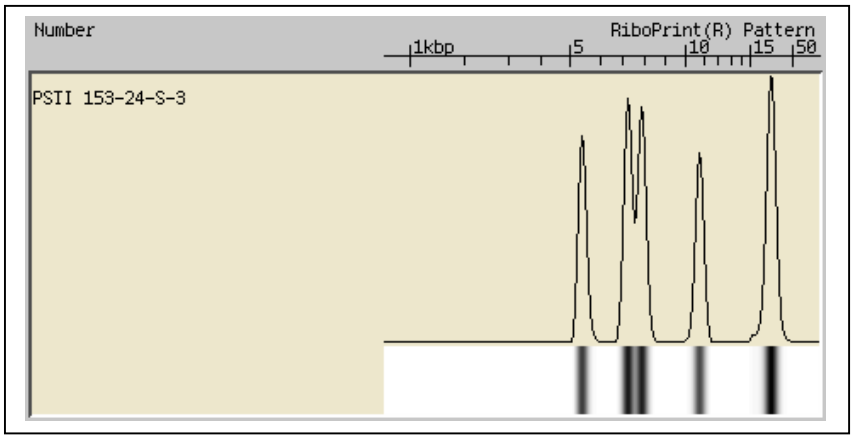
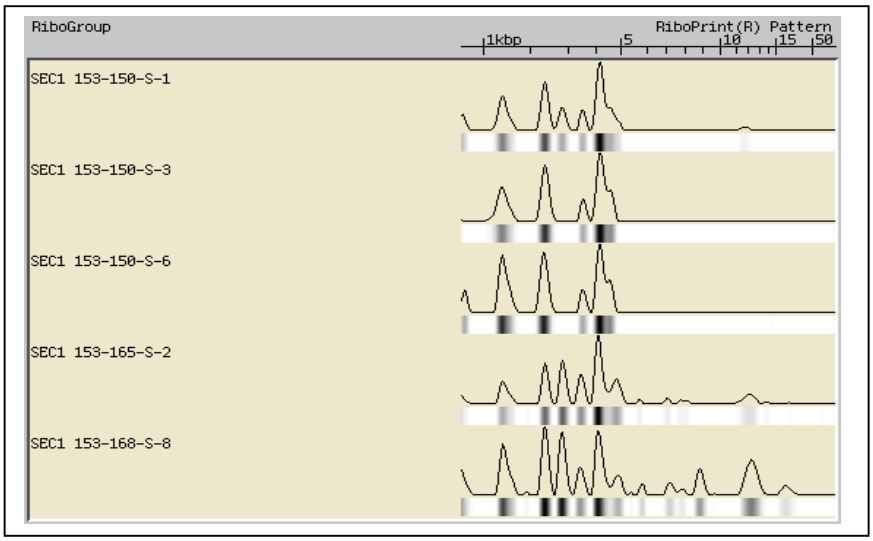


Figure 2 – *Hae*III ribotyping profiles identified among the *C. coli* isolates



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INFECTIONS CAUSED BY CONDITIONALLY - PATHOGENIC MICROORGANISMS.

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Keywords : poultry meat, conditionally pathogenic microflora.

Abstract

We have established specific structure selected microflora: E.coli, Kl.pneumoniae, S.enteritidis, Pr.vulgaris, Ent.agglomerans, Citr.diversus, Hafnia alvei, Shigella boydii, Ps. aeruginosa, St. aureus, Streptococcus, Cl. perfringens. On the basis of the carried out(spent) researches we made conclusions:
- The infections of poultry caused conditionally – pathogenic microflora have a wide circulation in poultry - farms of Ukraine.
- The isolated cultures conditionally - pathogenic microflora irrespective of a place of their isolation (corpses, carcasses of poultry, air environment of poultry - farms objects) had pathogenic and toxigenic properties, and in this connection are the aetiological factor toxicoinfections at the man.

Résumé

Une enquête sur la présence de bactéries potentiellement pathogènes a été entreprise dans 48 élevages de volailles en Ukraine. Il a été démontré que les bactéries pathogènes circulaient largement dans les fermes ukrainiennes et que leurs propriétés toxinogènes et pathogènes pouvaient entraîner des risques de toxi-infections chez l'homme.

Introduction

Conditionally - pathological microorganisms and diseases, caused by them, one of urgent and difficult problems of an infectious pathology of the man, animals and

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poultry. The increasing alarm is caused by number of these diseases, which basic reason is wide and unsystematic application of antibiotics, and also preparations overwhelming protective forces of organism (corticosteroides, immunodepressing substance).Having high stability to antibiotics, is conditional pathogenic microorganisms frequently cause toxical infections at the man. To conditionally - pathogenical microorganisms, which role in a pathology of poultry is proved or is studied concerns: Escherichia coli, Edwardsiella tarda, Citrobacter freundii, Enterobacter aerogenes, Klebsiella pneumonias, Hafnia alvei, Serratia marcescens, Proteus vulgaris, Iersiniae enterocolitica, Erwiniae gerbicola, Pseudomonas aeroginosa, Staphilococcus aureus, Streptococcus faecalis

Material and Methods

We carried out the retrospective analysis of allocation conditionally - pathoginical microflora in 48 poultry - farms of Ukraine of a various technological direction Thus took into account results of bacteriological researches on E.coli, coccus microflora, and also on activators, which role in a pathology of poultry is unsufficiently investigated. There are: Proteus, Ps. aeruginosae, Klebsiella.

Results and discussion

Generalizing stated it is possible to conclude, that is conditionally – pathogenic microflora circulates in all poultry - farms of a various type and among all age groups the poultry. Isolation pathogenic organisms we carried out from the organisms sick and died poultry, from air environment poultry farms, from cases hatchery at the moment of a conclusion the poultry. Thus have established specific structure selected microflora:E.coli, Kl.pneumoniae, S.enteritidis, Pr.vulgaris, Ent.agglomerans, Citr.diversus, Hafnia alvei, Shigella boydii, Ps. aeruginosa, St. aureus, Streptococcus, Cl. perfringens. At study of pathogenic properties we have established, that the isolated cultures were pathogenic for white mice and 30-day's chickens. The similar cultures by us were isolated and from a poultry killed on sanitary shambles of poultry – farms. At study of toxigenic properties of the isolated cultures on rats and on ligatured loops intestine chickens we have established: 59 % of cultures had a high degree toxic, 28 % - weak degree at 13 % - cultures were absent e of toxigenic property. Studying quality of meat received from the sick poultry caused by infections conditionally – pathogenic microflora we have established, that such carcasses have smaller an slaughter output on 1,7 % in comparison with healthy poultry. Besides the

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quantity of water is increased. The quantity of fat decreases, is reduced calorie content of meat. Such carcasses are not subject to a storage, as are exposed to damage for the third day at a storage them at temperature 0- 4^0C. On the basis of the carried out(spent) researches we made conclusions: - the infections of poultry caused conditionally – pathogenic microflora have a wide circulation in poultry - farms of Ukraine.

- The isolated cultures conditionally - pathogenic microflora irrespective of a place of their isolation (corpses, carcasses of poultry, air environment of poultry - farms objects) had pathogenic and toxigenic properties, and in this connection are the aetiological factor toxicoinfections at the man. Meat of the poultry, received from the sick poultry has the raised quantity of a moisture on 1,77-2,27% reduced quantity of fat on 4,2 % and lowered kalorie content on 130-134 kJ. In this connection the carcasses of poultry, received from poultry - farms unsuccessful on infections caused conditionally – pathogenic microflora it is necessary necessarily to boil on the amplified mode, and the internal bodies are directed on recycling, at presence degeneretive changes in carcasses and carcasses and the internal bodies are exposed to recycling.

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CONTENTS

ANTIMICROBIAL SUSCEPTIBILITIES OF *CAMPYLOBACTER* STRAINS ISOLATED FROM CHICKEN CARCASSES IN SENEGAL

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Keywords: Antimicrobial resistance, Fluoroquinolone, *Campylobacter*, Africa

Abstract

To assess antibiotics susceptibility of 204 *Campylobacter* strains, collected from skin samples issued from 250 chicken carcasses (January 2001 to October 2002) in Senegal in vitro susceptibility to five antimicrobial drugs (amoxicillin, amoxicillin-clavulanic acid, erythromycin, nalidixic acid and ciprofloxacin) was determined by E test method. Thirty four percent of *Campylobacter* isolates were ciprofloxacin resistant with a high level of resistance (MIC ≥ 32 µg/ml). Cross-resistance between nalidixic acid and ciprofloxacin was found in 96% of quinolone resistant strains. The level of amoxicillin resistance was statistically higher for *C. coli* than for *C. jejuni* (20.2% versus 10.8%) but all the strains were susceptible to amoxicillin-clavulanic acid. The two species showed low resistance to erythromycin. Multiresistance phenotype to three of the drugs tested was found among 9.8% of the strains: 15.5% among *C. coli* strains and 5.8% among *C. jejuni* strains. No strain was resistant to four or more drugs studied. Further studies appear necessary to evaluate antibiotics resistance among *Campylobacter* isolated from human and animal samples in order to control the emergence of new multidrug resistance strains in Senegal.

Résumé

La résistance aux antibiotiques (amoxicilline, amoxicilline- acide clavulanique, erythromycine, acide nalidixique et ciprofloxacine) de 204 souches de *Campylobacter* isolées de 250 carcasses de poulet de Janvier 2001 à Octobre 2002 au Senegal a été déterminée par la méthode du E test. 34% des isolats résistaient à la ciprofloxacine à un niveau élevé (MIC ≥ 32 µg/ml). La résistance croisée acide

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nalidixique/ciprofloxacine intéressait 96% des isolats résistants aux quinolones. Le niveau de résistance à l'amoxicilline était plus élevé chez *C. coli* que chez *C. jejuni* (20.2% contre 10.8%) mais tous les isolats étaient résistants à l'amoxicilline-acide clavulanique. La résistance à l'érythromycine était faible pour les deux espèces. Les phénotypes multirésistants à 3 des antibiotiques testés intéressaient 15.5% des isolats de *C. coli* et 5.8% de *C. jejuni.* Aucune souche n'était résistante à plus de 3 des antibiotiques testés.

Introduction

In Senegal, a modern poultry production sector is expanding around Dakar, capital city, in order to rapidly supply the fast-growing urban human population with animal protein. Poultry farmers use antibiotics to control diseases. Growing scientific evidence that use of antimicrobial agents in veterinary medicine results in development of resistant pathogenic bacteria[1] leads us to assess the frequency of antimicrobial resistance of *Campylobacter* strains isolated from "modern" poultry meat.

Materials and methods

Specimen collection and isolation

From January 2001 to October 2002, 250 chicken carcasses were collected from slaughter houses and retail shops in Dakar regarding the ISO standards. Isolates were identified using both API Campy® and a multiplex PCR.[2]

Susceptibility testing

Several colonies of each strain were suspended in 5 ml of Mueller-Hinton broth to achieve a turbidity equal to 0.5 Mac Farland standard. The suspensions were inoculated with sterile swabs onto Mueller-Hinton agar with 5% sheep blood. After application of E-test® strips (AB Biodisk – Sweden) plates were incubated at 37°C for 48 h under microaerophilic atmosphere. The MICs were read according to the recommendations of the manufacturer by 2 different readers.

The breakpoints were those recommended by the Antimicrobial Committee of the French Society for Microbiology[3]. Breakpoints for resistance susceptibility were respectively >16 mg/l for amoxicillin, >16/2 mg/l for amoxicillin-clavulanic acid, >4 mg/l for erythromycin, >16 mg/l for nalidixic acid and >2 mg/l for ciprofloxacin.

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Results

The resistance rate to amoxicillin in *C. coli* and *C. jejuni* was 20.2% and 10.8% respectively. Among *C. coli*, all resistant strains had a MIC > 256 mg/l. Among *C. jejuni*, 46% of the amoxicillin resistant strains had a MIC >256mg/l. In addition, amoxicillin MIC_{90} is statistically higher for *C. coli* than for *C. jejuni*.

Regarding amoxicillin-clavulanic acid, no resistant strains were observed. For amoxicillin high level resistant strains (MIC > 256 mg/l), Amoxicillin-clavulanic acid MIC was between 2/1 and 8/4 mg/l in both species.

Overall resistance rate to erythromycin was 4.7% for *C. coli* and 3.3% for *C. jejuni* without any significant difference between the 2 species.

The resistance rate to nalidixic acid in *C. coli* and *C. jejuni* was respectively 34.5% and 31.6%. Among these resistant strains, 55.2% of *C. coli* and 68.5% of *C. jejuni* exhibited a MIC>256 mg/l. For both species, MIC_{90} was >256 mg/l.

Compared to nalidixic acid, quite similar resistance rates to ciprofloxacin were observed with respectively 34.5% and 30.8% for *C. coli* and *C. jejuni*. Ciprofloxacin MIC was >32 mg/l for 25% isolates in both species. Among *Campylobacter* resistant strains, 75.8% of *C. coli* and 78.3% of *C. jejuni* had a ciprofloxacin MIC>32mg/l. 90% of the isolates were inhibited with a MIC>32 mg/l for *C. coli* and *C. jejuni.* For both species, MIC_{90} was >32 mg/l.

Co-resistant and Multiresistant isolates

Drug resistance to one or more drugs was detected in over 38% of the strains : Among *C. coli* and *C. jejuni*, respectively 41.7% and 36.7% of the strains exhibited a resistance to the tested antibiotics.

Among *C. coli* and *C. jejuni*, 33.4 % and 30 % were respectively resistant to both quinolones (nalidixic acid and ciprofloxacin). A cross-resistance between nalidixic acid and ciprofloxacin was found for all isolates (96%) except one strain of *C. jejuni* and one strain of *C. coli* which showed a nalidixic acid–resistant ciprofloxacin–susceptible phenotype. 45.6% of *C. coli* strains and 63.8% of *C. jejuni* strains exhibited a high level of resistance to both quinolones with MIC>256 mg/l for nalidixic acid and MIC>32 mg/l for ciprofloxacin.

Multiresistance, defined as resistance to three or more of the drugs tested, was found in 9.8% of the *Campylobacter* strains: 15.5% were *C. coli* strains and 5.8% were *C. jejuni* strains. No strain was resistant to four or five drugs. The only multidrug resistance phenotype was amoxicillin, nalidixic acid and ciprofloxacin in both species.

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Discussion

Because no great discrepancies have been observed between double dilution Agar and E test according the reports of Baker[4] and Funke[5], we used E test for susceptibility testing. This method is reliable and is also technically simple and needs no special equipment[5].

In our survey, high resistance rates to quinolones were observed in both species isolated from chicken samples. This important resistance rate to quinolones was similar as those observed in several European countries[6] or in Japan[7]. As reported by Aarestrup *et al.* in Denmark[1], a cross resistance between nalidixic acid and ciprofloxacin was observed. But unlike Thwaites and Frost in United Kingdom[8], *Campylobacter* strains with a high level of resistance to both quinolones were predominant.

Nevertheless, rates of resistance to ciprofloxacin observed in our study appeared lower than those described in Belgium[9], or Lebanon[10] where resistance rates ranged from 61% to 100%. By contrast, no *Campylobacter* strains were found ciprofloxacin resistant in Chile [11].

Since 1991, when Endzt and colleagues[12] identified the first quinolone resistant *Campylobacter* strains in *C. jejuni* and *C. coli* in the Netherlands, *Campylobacter* resistance to FQ has gone to increase throughout the world. Actually, the percentage of ciprofloxacin-resistant strains in humans has been increasing in Spain from 0-3 % in 1989 to 30 – 50 % in 1991 after the licensing of enrofloxacin in 1990 for animal husbandry.[13] During this period, the same evolution has been observed in Finland[14] and Canada.[15] Likewise, in England, before the introduction of FQ in veterinary medicine, a study showed that domestically poultry bred were less contaminated by ciprofloxacin resistant strains than those imported from countries where the use of FQ was legal.[16] Thus, this important development of FQ resistance strains in humans and animals seems related to the introduction of FQ in veterinary medicine.[12]

In Senegal, FQ (norfloxacin, enrofloxacin) were introduced in veterinary medicine in 1996 in poultry production to treat respiratory and intestinal diseases as salmonellosis or colibacillosis. And since 2000, because of failures of treatment with other antibiotic drugs, they became the first line molecules. Thus, according observations from other reports and in spite of any previous studies on antibiotic resistance in Senegal, the high prevalence of FQ resistance could be related to the introduction of FQ in the country, especially for poultry production.[1] And, as in developed countries, FQ resistance may become soon a public health problem

We found high levels of amoxicillin-resistance, particularly in *C. coli* strains. *Campylobacter* resistance to penicillin A has been reported by previous studies with resistance rate never exceeding 39%.[1,11,17] However, in Senegal, amoxicillin was used rarely as treatment against pasteurellosis or salmonellosis in poultry production.

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Resistance is usually associated with β-lactamase production but other mechanisms of resistance could be involved, such as modified penicillin-binding proteins or impermeability.[18] As reported in other studies most of our isolates showed high susceptibility to amoxicillin-clavulanic acid.[10]

Among strains isolated, 4.7% of *C. coli* strains and 3.3% of *C. jejuni* strains isolated were resistant to erythromycin. This appeared much lower than results from Nigeria where resistance rate have reached 40.4% among animals strains.[19] In our study, erythromycin-resistance rate was similar for both species, contrary to the results from surveys conducted in Spain (respectively 17% and 83% of resistance in *C. jejuni* and *C. coli*)[18] or in Vietnam (respectively 17% and 50% of resistance in *C. jejuni* and *C. coli*).[20] This difference may be explained by the introduction, in these countries, of tylosin especially used as growth promoter in pig industry. In Senegal, macrolides were not used as growth promoters in poultry production but they are only used for treatment of some respiratory diseases like chronic respiratory disease.

For the five most relevant antimicrobial drugs selected for *Campylobacter*iosis treatment, seven resistance patterns were observed but only 9.8% of the total strains were resistant to three drugs. None were resistant to four or more drugs. Our results were much lower than results from Belgium[9] or Spain[10]. However, consistently with these studies, in our survey *C. coli* exhibited more multidrug resistant strains than *C. jejuni*.

Our study is the first to highlight the decrease of antibiotic susceptibility of *Campylobacter* isolated from chicken in Senegal. There are many reasons for explaining this situation including: 1) Lack of information among poultry farmers and veterinary surgeons about antibiotic use and drugs resistance, 2) Inordinate use of antibiotics due to the high prevalence of infectious diseases among flocks, 3) Difficulties to perform large antimicrobiological studies.

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CONTENTS

THE SURVEY OF FREQUENCY OF CAMPYLOBACTER JEJUNI IN LIVER BROILERS CHICKENS IN SLAUGHTER HOUSE & RETAIL MARKET BROILERS IN SHAHREKORD, IRAN

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Keywords: Liver broilers,C.jejuni,Slaughter house,Retal market.

Abstract

This survey was conducted on 400 liver samples in order to detection of Campylobacter jejuni in liver of broilers chickens during summer 2001 to spring 2002 in Shahrekord township.All of the samples were cultured in enrichment and specific bacteriological media for C.jejuni.Out of 400 samples,259 samples(64.75%) were positive due to C.jejuni.From 200 samples taken from slaughter house 129(64.5%)and from 200 samples taken from retail markets,130(65%) were positive. In comparison of the infection rate in different seasons,autumn 2001 with 80(80%) was highest rate and spring 2002 with 45(45%) was lowest rate.Therefore the consumption of infected livers to C.jejuni constitutes a major health hazard for human in this city.

Résumé

Cette étude a permis de surveiller la contamination de 400 foies de volailles par campylobacter entre l'été 2001 et le printemps 2002 dans la ville de Shahrekord. 259 échantillons parmi les 400 (64,75%) se sont avérés positifs pour c.jejuni, 129 parmi les 200 échantillons issus d'abattoirs (64,5%) et 130 parmi les 200 échantillons de produits prélevés dans les points de vente (65%). La contamination maximale a été observée en automne 2001 (80% de positifs), tandis que les minima ont été enregistrés au printemps 2002 (45% de positifs). La consommation de foie de volailles contaminés par campylobacter constitue un risque pour le consommateur.

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Introduction

Campylobacter is an important human pathogen and has been estimated to cause more than 2,000,000 cases of food borne illness a year(1).The infection by C.jejuni causing of diarrhea in industrial contries is a main problem of public health and economic burden(2).Campylobacter takes the second place after salmonella among the bacterial enteritic pathogens(3,5).In some contries such as the UK,Campylobacter species were more frequently isolated from diseased humans than salmonella(1).one important reservoir of C.jejuni is birds and poultry,between 50% and 80% of flocks of broilers arriving at the slouther houses are healthy carriers of C.jejuni(6,7) .C.jejuni was isolated mainly from the skin,breast muscls,offal(liver,heart and stomach) broilers(4).In order to prevent C.jejuni contamination of poultry and control the hygiene,we realised the frequency of C.jejuni in apperently normal of livers broiler in slaughter house and retail market broilers in Shahrekord,IRAN.

Material and methods

During a years period from summer 2001 to spring 2002, culture of the total 400 normal livers samples obtained from slaughtered broiler chickens and various retail market broilers in shahrekord township,were cultured for C.jejuni by direct plating on a selective medium after enrichment techniques were used.Therefore 2 gram of liver stomachered for one minute and was taken out in the enrichment media Stuart transport agar (MERCK,Germany) and incubated for 24 hours at 42□C .After 24 hours a loopful of enrichment broth was plated on Campylobacter selective Agar(MERCK, Germany ,2248) with Campylobacter selective supplement(MERCK, Germany ,2249) and 5% sheep defibrinated blood and incubated for 48-72 hours, 42□C under micro-aerophilic conditions(5%O2 , 10% CO2 and 85% N2).Colonies resembling campylobacter were Gram-stained and oxidase and catalase tests were performed. Further differentiation of campylobacter species wasd carried out using hippurat hydrolysis and sensitivity to nalidixic acid and cephalothin.All samples were compared by analysis of variance with using statistic soft ware(sigma stat).

Results

Out of 400 apperently health livers broiler samples,259(65.75%) samples were positive for C.jejuni. Of 200 samples livers obtained from slaughter house broiler

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chicken,129 (65%) and of 200 samples livers obtained from retail market broilers 130(65%),were positive.Also maximum number of isolates was been in autumn months 2001 (80%) and spring months 2002 with 45% was lowest rate.A significant difference in the total frequency of C.jejuni with season by analysis of varience($p<0.001$).
Tables 1 &2 shows results of C.jejuni detection in liver broiler.

Table -1) Detection of C.jejuni in liver broiler in Shahrekord,IRAN.

Samples place	Number of positive samples	Number of negative samples
Retail market	130(65%)	70(35%)
Slaughter house	129(64.5%)	71(35.5%)

*Not was seen significant difference between samples of retail market & slaughter house by analysis of varience ($p<0.001$).

Table-2) Detection of C.jejuni in liver broiler in different seasons (2001-2002) in Shahrekord, IRAN.

Sample Season	Positive	Negative
Summer 2001	71(71%)	29(29%)
Autumn 2001	80(80%)	20(20%)
Winter 2001	63(63%)	37(37%)
Spring 2002	45(45%)	55(55%)

*A significant difference in the frequency of C.jejuni with season by analysis of varience ($p<0.001$).

Discussion

Poultry is the most commonly reported sources of campylobacter infections in many countries(8).In a total of 400 samples of livers broiler in Shahrekord,64.75% were positive for C.jejuni.The results of this study show that a large number of liver broiler are infected in this area.The seasonality has been suggested to be related in broiler chicken with C.jejuni.In a Dutch one year study of 187 broiler flocks at slaughter,Campylobacter carriage was highest(100%) during period june- september and lowest(50%) in march(Jacops-Reitsma,1994).In this study ,the infection rate in this seasons,autumn 2001 with 80 sample(80%) was highest rate and spring 2002 with 45(45%) was lowest rate.In a study in Norway,the proportion of colonised broiler flocks peaked in the autumn,i.e. after the summer peak63% of campylobacter posiyive

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flocks were detected during august to november(Kapperad et al,1993).Humphrey et al(1993) did not find any apparent seasonal variation in campylobacter presence in 49 flocks at slaughter between june1990 and july1991.
Therefore are a potential source of C.jejuni infection for people in this city.

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QUANTIFICATION OF CAMPYLOBACTER THERMOPHILIC IN ITALIAN POULTRY PRODUCTS

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Keywords: Campylobacter thermophilic, MPN, poultry meat products

Abstract

The aim of the study was to investigate the survival of Campylobacter thermophilic in fresh broiled chicken and skewer chicken products, packaged in ordinary and modified atmosphere, during the refrigerated storage at 4°C. The quantification of Campylobacter was performed by using the most probable number. The Campylobacter initial load detected in the broiled chickens packaged in ordinary and modified atmosphere was five and eight times higher than in skewer chickens packaged in the same way. In broiled chicken products packaged in ordinary as well as modified atmosphere Campylobacter decreases during the first three days of storage but then increases again. In the skewer chicken samples the Campylobacter load detected was initially low and did not increased on time.

Résumé

L'objectif de cette étude était d'étudier la présence de Campylobacter thermophile dans le poulet rôti et les brochettes de poulet emballés en atmosphère ordinaire et modifiée pendant leur conservation à 4°C. La quantification de Campylobacter a été effectuée en utilisant la méthode du nombre plus probable. Le niveau initial de Campylobacter, détecté dans le poulet rôti emballé en atmosphère ordinaire et modifiée, était cinq et huit fois plus haut de celui détecté dans les brochettes de poulet emballées de la même façon. Dans le poulet rôti aussi bien emballé en atmosphère ordinaire que modifiée, Campylobacter décroît dans les trois premiers jours d'entrepôsage pour augmenter ensuite à nouveau. Dans les brochettes de poulet le niveau de Campylobacter identifié était initialement bas et n'a pas augmenté avec le temps.

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Introduction

Contaminated animal products, particularly poultry meat, are recognised as being the primary vehicles of Campylobacter infection since this pathogenic bacteria frequently occurs in poultry (Shane, 2000). Different studies carried out in Italy have demonstrated the presence of Campylobacter in poultry and poultry products (Pezzotti et al., 2003); however only partial data are available at the moment. The aim of this study was to investigate the Campylobacter thermophilic contamination level in two different kinds of fresh poultry meat products and the survival of this pathogenic bacteria during the refrigerated storage at 4°C in ordinary and modified atmosphere.

Material and Methods

The poultry meat products tested in this study were represented by raw broiled chicken and skewer chicken, packaged in ordinary (OA) and modified (MA) atmosphere. The modified atmosphere was made up of 80% oxygen; 10% nitrogen; 10% carbon dioxide. Campylobacter was quantified by using the most probably number (MPN) technique. The chicken products were collected in eleven different days along seventy working days during the winter. At each sampling day three samples of each chicken meat product were collected. One of those was tested immediately, whereas the other two after three and eight days of storage at 4°C. At each sampling time, ten grams of different parts of each product were added to 90 ml of buffered peptone water and gently homogenised by hand for 5 minutes. Then, 1 ml of the suspension obtained was decimally diluted three times. Three aliquots of one ml each were obtained from each dilution set and added in three tubes containing 9 ml of Campylobacter Selective Enrichment broth (Oxoid). All tubes were then incubated at 42°C for 24 h in modified atmosphere, obtained using CampyGen (Oxoid). Following incubation, one loopful from each tube was streaked onto mCCDA (Oxoid) plates, incubated in modified atmosphere at 42°C for 48 h. Campylobacter suspected colonies were subcultivated until monocultures were obtained. Curved or spiral Gram negative rods, catalase and oxidase positive were presumptively identified as Campylobacter.

Results and Discussion

The mean values of Campylobacter contamination in the four types of poultry meat products examined are shown in Table 1. The Campylobacter load detected in the

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broiled chickens packaged in ordinary and modified atmosphere was five and eight times higher than in skewer chickens packaged in the same way. This result might be explained considering that the first kind of product contains chicken skin and more fat in comparison to the skewers.
In Table 2 are shown the mean values of the Campylobacter contamination level detected in the four poultry products at the production time and after three and eight days of storage at 4°C. The Campylobacter load detected in the broiled chickens decreases during the first three days but then increases again in ordinary as well as modified atmosphere. The Campylobacter load detected in skewer chickens was initially low and did not increased on time.
In Figure 1 is shown the survival of Campylobacter in each of the eleven sample of broiled chicken packaged in modified atmosphere analysed. The initial Campylobacter load was very different among samples and the standard deviation calculated was 0.91. A very high variability was also observed for the same type of product packaged in ordinary atmosphere (Figure 2). For these samples a very high standard deviation was observed at 0 days (i. e. 0.98) as well as after three (i. e. 1.62) and eight (i. e. 1.67) days of storage. A lower standard deviation was observed for the skewer chickens packaged in modified (Figure 3) and ordinary (Figure 4) atmosphere. In fact the initial Campylobacter load of this products was always less than 1 log MPN/g and any positive samples was detected after 8 days of storage.
Conclusions: 1) the Campylobacter contamination level is significantly different in different poultry meat products; 2) the packaging conditions do not significantly influence the Campylobacter survival and, in particular, the presence of oxygen does not inihit this pathogenic bacteria, expecially when the product contains fat and skin (i. e. broiled chicken); 3) Campylobcter can easly survive at the refrigeration temperatures for more than eight days, that normally represent the shelf life of fresh poultry meat products in Italy.

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Table 1- Means Log MPN/g calculated in the four types of meat products tested

Meat product	N. samples	Log MPN/g (mean ± stand. dev.)
Broiled chicken OA	33	$1,54 \pm 1,07^{b}$
Broiled chicken MA	33	$1,75 \pm 1,01^{b}$
Skewer chickens OA	33	$0,33 \pm 0,47^{a}$
Skewer chickens MA	33	$0,21 \pm 0,47^{a}$

Table 2 - Means Log MPN/g calculated in the four types of meat products tested during their storage at 4°C

Meat product	Days of storage	N. samples	Log MPN/g (media ± stand. dev.)
Broiled chicken OA	0	11	$1,96 \pm 0,98^{ab}$
	3	11	$1,62 \pm 1,05^{abcd}$
	8	10	$1,67 \pm 1,25^{abc}$
Broiled chicken MA	0	11	$2,35 \pm 0,91^{a}$
	3	11	$1,12 \pm 0,75^{abcde}$
	8	11	$1,14 \pm 0,90^{abcde}$
Skewer chickens OA	0	11	$0,60 \pm 0,53^{bcde}$
	3	11	$0,39 \pm 0,48^{cde}$
	8	11	0^{e}
Skewer chickens MA	0	11	$0,46 \pm 0,43^{cde}$
	3	11	$0,19 \pm 0,34^{de}$
	8	11	0^{e}

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Figure 1 – Campylobacter survival in broiled chicken packaged in modified atmosphere

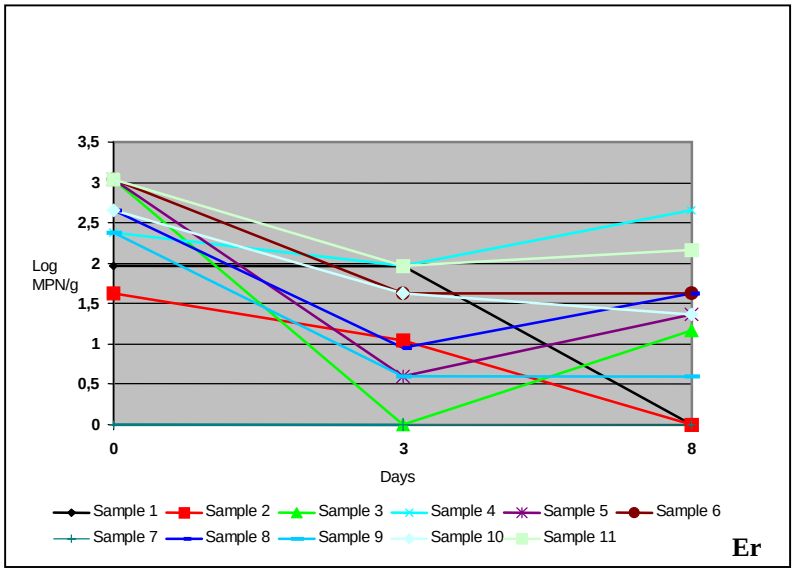

Figure 2 – Campylobacter survival in broiled chicken packaged in ordinary atmosphere

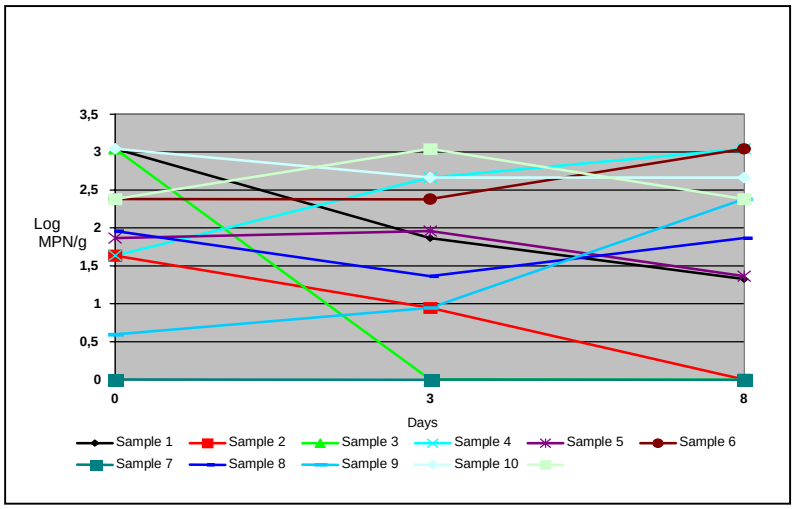

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Figure 3 – Campylobacter survival in skewer chickens packaged in modified atmosphere

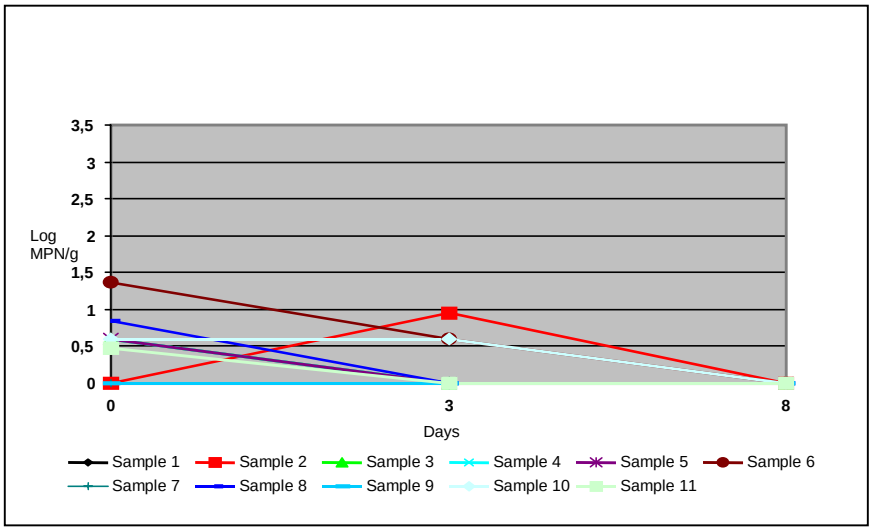
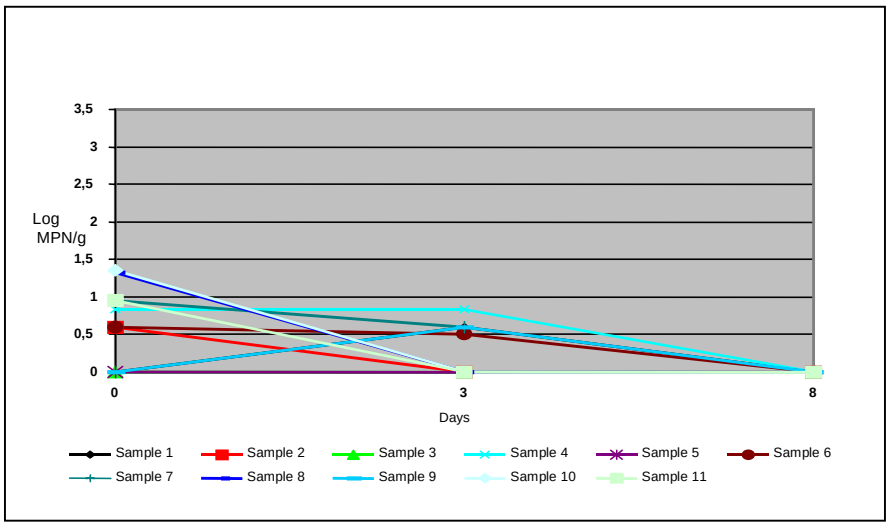


Figure 4 – Campylobacter survival in skewer chickens packaged in ordinary atmosphere



CONTENTS

ON-LINE ANTIMICROBIAL REPROCESSING OF PRE-CHILL BROILER CHICKEN CARCASSES

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Keywords: Broilers, on-line reprocessing, antimicrobial, TSP

Abstract

On-line reprocessing involves treatment of carcasses contaminated with visibly with digestive tract contents with an antimicrobial prior to chilling. In this field study, the effectiveness of trisodium phosphate (TSP ; Assur-Rinse^{TM}), applied pre-chill as a 8-12% solution in a spray/drench cabinet) was assessed under commercial broiler processing conditions. TSP was effective ($P<0.05$) in reducing the *Escherichia coli* and total coliform counts and *Salmonella* incidence on carcasses that were visually contaminated. Inspite of pH adjustment, microbial recovery was significantly affected by frozen storage of carcass rinse samples (18-24h). Although microbial recovery rates were higher post-chill than immediately after TSP application, on-line reprocessing with TSP was found to be an effective food safety intervention step during processing.

Résumé

Le retraitement des carcasses en ligne consiste à appliquer un traitement chimique de décontamination pour les carcasses de volailles présentant des souillures fécales rompt le refroidissement. Dans cette étude de terrain, l'efficacité du phosphate trisodique (TSP, AssurinseTM) à 8-12% en pulvérisation a été évaluée dans des conditions industrielles. Le TSP s'est avéré efficace sur E coli, les coliformes totaux et la contamination par salmonella sur les carasses souillées visuellement. Malgré la neutralisation du liquide de rinçage de la carcasse, les démembrements microbiens sont affectés par la congélation du prélèvement (18-24h). Bien que les démembrements bactériens soient plus élevés après le refroidissement

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qu'immédiatement après le traitement au TSP, la méthode est considérée comme un moyen efficace de réduction du risque sanitaire sur les carcasses souillées.

Introduction

A number of food safety performance standards have been introduced for the meat and poultry processors in the US, as essential components of the Hazard Analysis and Critical Control Points (HACCP) system (USDA, 1996). In addition to monitoring levels of *Escherichia coli* and incidence of *Salmonella* on post-chill carcasses, a zero-tolerance standard for visible fecal contamination is also mandated. This ruling is based on the assumption that fecal material is the primary source and vehicle for food-borne pathogens on poultry carcasses that must be removed prior to immersion chilling to prevent cross-contamination. Carcasses accidentally contaminated with fecal material are typically removed from the main processing line during carcass inspection, reprocessed off-line by trimmimg, vacuuming and washing with chlorinated (20 ppm) water, and reinspected prior to chilling. Several studies have shown that off-line reprocessed carcasses were microbiologically indistinguishable from visually clean, inspection passed carcasses (Blankenship *et al.,* 1975, 1993 ; Bilgili *et al.,* 1992 ; Fletcher *et al.,* 1997 ; Waldroup *et al*., 1993).
Several antimicrobial compounds, including trisodium phosphate (TSP), acidified sodium chlorite, and peroxyacetic acid, have been developed and marketed to treat to all pre-chill eviscerated carcasses, with or without visible contamination, as part of a on-line reprocessing system. The objective of this study was to evaluate the effectiveness of TSP (Assur-Rinse^{TM}) on-line reprocessing system under commercial conditions.

Material and Methods

Three experiments (Exp) were conducted in cooperation with commercial broiler plants in the US utilizing on-line, pre-chill TSP antimicrobial system (8-12% solution). In Exp.1, microbiological quality of visibly clean, inspection passed (VC), visibly contaminated, on-line TSP reprocessed (FOR), and visibly contaminated, off-line reprocessed (FR) carcasses were microbiologically compared in six plants (n=2400). In Exp.2, microbiological quality of carcasses were compared before (B-TSP) and after the TSP (A-TSP) spray cabinet and after immersion chilling (A-C) in nine plants (n=3600). Both in Exp.1 and 2, microbial

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recovery was also compared in rinsates taken after TSP application (FOR and A-TSP treatments, respectively), held either frozen or refrigerated (4C) for 18-24 h during shipment to the laboratory. In Exp.3, the microbiological quality of carcasses (n=30) were also compared after TSP application (A-TSP) and after immersion chilling carcasses individually in tubs for 70 min at 4C (A-IIC).
Carcass were sampled aseptically and rinsed with 400 ml of Butterfield's Phosphate Buffer Solution based on USDA (1996) sampling protocol. The pH of the rinsate was adjusted to 6.8 +/- 0.2 with 0.2N HCl or 0.1N NaOH. Rinsates were shipped overnight to an analytical laboratory, appropriately diluted and plated for total coliforms and *E. coli* incidence (%) and counts (CFU/ml), as well as *Salmonella* spp. incidence (%) by methods described by the AOAC (2000). All quantitative data was analyzed by the General Linear Models procedure of SAS (1988). The statistical model included plants as blocks, main effects of treatment, site and their interaction. Chi-Square tests were performed to compare *Salmonella* incidence betwenn the treatments at each site and between each site. Level of significance was set at $P<0.05$.

Results and discussion

E.coli, total coliform, and *Salmonella* recovery rates (incidence and counts) in Exp.1 are summarized in Figures 1-2. Carcasses treated with TSP (FOR) had lower incidence of *E.coli* and *Salmonella*, and lower counts of *E.coli* and total coliforms than VC and FR treatments. The TSP effectiveness was also confirmed on carcasses immediately after the application cabinet (A-TSP) in Exp.2 (Figure 3-4). Although *E.coli* and total coliform counts remained lower than the pre-TSP application levels through the immersion chilling system, microbial populations tended to increase post-chill. As compared to the refrigerated storage, freezing carcass rinse samples during overnight shipment to the laboratory negatively affected the recovery of test microorganisms (Figures 5-7). Exp.3 was conducted to investigate the reason for the slight increase observed in microbial populations after chilling in Exp.2 (A-IC treatment). Individually chilling carcasses eliminated the chance of cross-contamination during immersion. However, as observed before, microbial recovery rates increased significantly following chilling as compared to the post-TSP application levels (Figures 8-9).
The antimicrobial properties of TSP is attributed to removal of fat and consequently to detaching effect on contaminants from the skin, as well as to its high pH (Lillard, 1994). Since high pH (about 12) of TSP treatments could interfere with the microbial recovery, carcass rinse solutions must be immediately neutralized (Tamblyn *et al.,*1997). In this study, on-line TSP application was

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effective in reducing, but not totally eliminating, the level and incidence of microorganisms tested. In spite of pH neutralization, the microbial recovery rates were significantly affected by freezing carcass rinse samples. Increasing the enrichment period for an additional 24 h increased *Salmonella* recovery rate, but not to the level obtained from the refrigerated rinse samples (Figure 10). It is clear from these results that, TSP interferes with the recovery of microbial populations. Recovery rates in this study were significantly affected by post-TSP freezing and immersion chilling, most likely due to sublethal injury. Overall, on-line reprocessing of visibly contaminated carcasses with TSP prior to chilling was found to be an effective food safety intervention step.

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Figure 1. *Salmonella* and *E.coli* incidence (Exp.1)

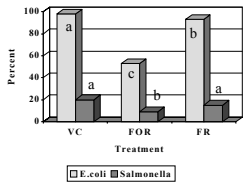
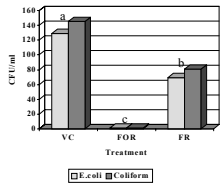


Figure 2. *E.coli* and total coliform counts (Exp.1)



P<0.05

Figure 3. *E.coli* and *Salmonella* incidence (Exp.2)

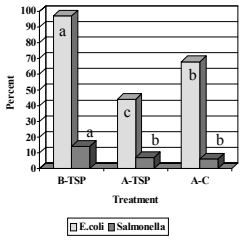


Figure 4. *E.coli* and total coliform counts (Exp.2)

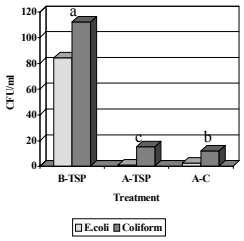


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Figure 5. *E.coli* and total coliform counts on refrigerated and frozen samples (Exp. 1)

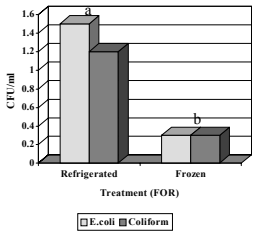


Figure 6. *E.coli* and total coliform counts on refrigerated and frozen samples (Exp. 2)

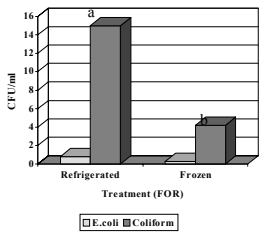


Figure 7. *E.coli* and *Salmonella* incidence on refrigerated and frozen samples (Exp. 2)

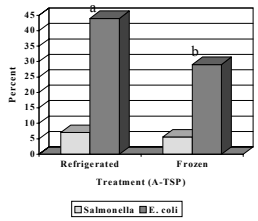


Figure 8. *E.coli* and total coliform incidence (Exp. 3)

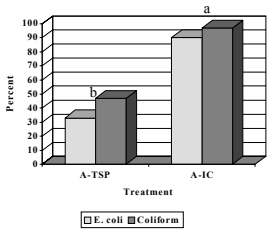


Figure 9. *E.coli* and total coliform counts (Exp.3)

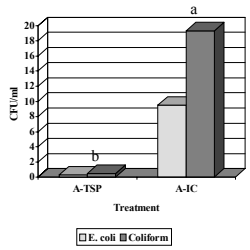
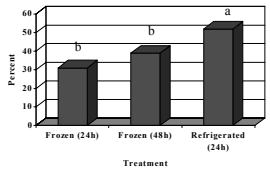


Figure 10. Post-TSP *Salmonella* recovery: Effect of storage temperature and enrichment time



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MOLECULAR TYPING OF *SALMONELLA ENTERICA* SUBSP. *ENTERICA* SEROVAR ENTERITIDIS ISOLATES FROM MAN AND POULTRY

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Keywords: *Salmonella enterica*, epidemiological study, poultry

Abstract

Salmonellosis is the most frequent food borne toxi-infection in Croatia in recent years. Epidemiological investigations with molecular typing methods is necessary, because biochemical and serological diagnostic methods are not enough reliable to establish differences among isolates. Altogether 40 different strains of *S.* Enteritidis from man and poultry were analyzed regarding phage type, plasmid content and restriction pattern of the ones containing plasmids. Analyzing plasmid profile of human and chicken *S.* Enteritidis strains phage type 9a did not reveal significant differences. Restriction pattern *Eco*RI included two fragments of about 25 kpb and 2.51 kpb in size showed differences between strains according to origin.

Résumé

Les salmonelles sont la principale cause de toxi-infection alimentaire en Croatie. Les recherches épidémiologiques utilisant les méthodes moléculaires et de typage sont nécessaires parce que les méthodes diagnostiques biochimiques et sérologiques ne sont pas suffisamment sûres pour faire la distinction parmi plusieurs isolats. 40 souches de S.Enteridis isolées de l'homme et des volailles ont été typées par lysotypie, analyses du contenu plasmidique et analyse des profils de restriction plasmidiques. Aucune différence significative entre les souches du lysosome 9a d'origine humaine ou animale n'a pu être mise en évidence. Les profils de restriction par Eco-R1 ont permis d'isoler deux fragments de 25 kpb et 2,51 kpb selon l'origine de la souche.

CONTENTS

Introduction

Salmonellosis is the most frequent food borne toxi-infection in Croatia in recent years. *Salmonella* infections appear to be one of the most typical examples of an enteric disease that is transmitted from poultry meat and eggs to humans. *Salmonella* infections are a serious medical and veterinary problem world-wide and cause great concern in the food industry (Kelly *et al*., 2002). In humans can produce symptoms ranging in severity from intestinal disturbances to death (Nolan *et al.*1991). Two salmonella serotypes are the most important cause of human food poisoning and also cause disease in poultry, *Salmonella enterica* subsp. *Enterica* serovar Enteritidis (*S.* Enteritidis) and *S.* Typhimurium, although on rare occasions other serotypes such as *S.* Hadar*, S.* Berta*, S.*Virchow may cause disease in birds. Many incidents have been reported to be caused by *S.* Enteritidis (Barrow, 1989; Rodrigue *et al*., 1990). It is the most frequently isolated serotype in Europe, as well as in America. Many cases of this disease have been associated with eating raw or undercooked eggs. Broilers are also a considerable reservoir of infection for man (Lax *et al*., 1995).
Because of more successful control and possible eradication of the disease, one of the major duties of a microbiologist and epidemiologist is to differentiate pathogenic from apathogenic strains and to discover ways of infection. Biochemical, serological and phage typing diagnostic methods of *Salmonella* infections in man and animals are traditional (Tacket, 1989). Unfortunately they are not enough reliable to establish differences among isolates and can not satisfactory serve in epidemiological study (Borrego *et al,* 1992; Helmuth *et al.,* 1990). Genotyping analysis of the *Salmonella* by plasmids and chromosome DNA restriction profiles (RFLP) has been widely used (Millemann, 1998). Various molecular typing methods for the analysis of zoonotic bacterial pathogens have been developed during the last decade.

Material and Methods

Altogether 40 different strains of *S* Enteritidis from man in case of human poisoning and simultaneously from poultry (meat and eggs) were analyzed regarding phage type, plasmid content and restriction pattern of the ones containing plasmids.
Bacterial strains were biochemically confirmed by using commercial biochemical test the API20E system (Bio-Merieux, France) and were serologically characterized by the slide agglutination test with Salmonella polyvalent O and group H antisera (Institute of Immunology, Zagreb, Croatia). The strains were identified as *S.* Enteritidis (20 human and 20 chicken isolates).

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Phage typing: For phage typing, 10 different bacteriophages (donated generously be Central Public Health Laboratory, Colindale, London, UK) were used. The method of Ward et al. (1987) was performed.

Plasmid profile analysis: Plasmids harbored by Salmonella were extracted by the method of Birnboim and Doly (1979). They were separated in horizontal 0.8% (wt/vol) agarose gels in 1xTAE buffer at 80V for 1h and visualized by staining with 0.5 mg of ethidium bromide per ml.

Plasmid fingerprinting: Total plasmid DNA was digested with *Eco*RI (Boehringer Mannheim Biochemicals, Germany) according to the manufacturer's recommendations. There was no differences obtained pattern for human *S.* Enteritidis strains. So restriction enzyme digests of several strains were coprecipitated (0.3 M Na-acetate, 2V EtOH, incubated 30 min at –70 C, centrifuged at 13000g, 30 min at +4 C) and subsequently electrophoresed in a 0.8% (wt/vol) agarose gel in 1xTAE buffer at 80V for 1h. They were visualized by staining with 0.5 mg of ethidium bromide per ml. The same was used for *S*. Enteritidis isolated from chicken.

Results and discussion

Table 1. Phage types in *Salmonella* Enteritidis strains from Croatia.

PHAGE TYPES	HUMAN	POULTRY	TOTAL
9a	4	6	10
8	4	4	8
20a	5	2	7
23	2	3	5
4	1	4	5
21	1	1	2
12	1	-	1
Not typeable	2	-	2
TOTAL	**20**	**20**	**40**

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Figure 1.

1. *λ/Hind*III (10µl,250 ng/ µl)
2. *S.* Enteritidis PT 9a *(*chicken meat)
3. *S.* Enteritidis PT 9a (eggs)
4. *S.* Enteritidis PT 9a (eggs)
5. *S.* Enteritidis PT 9a (human)
6. *S.* Enteritidis PT 9a (human)
7. *λ/Hind*III (10µl,250 ng/ µl)

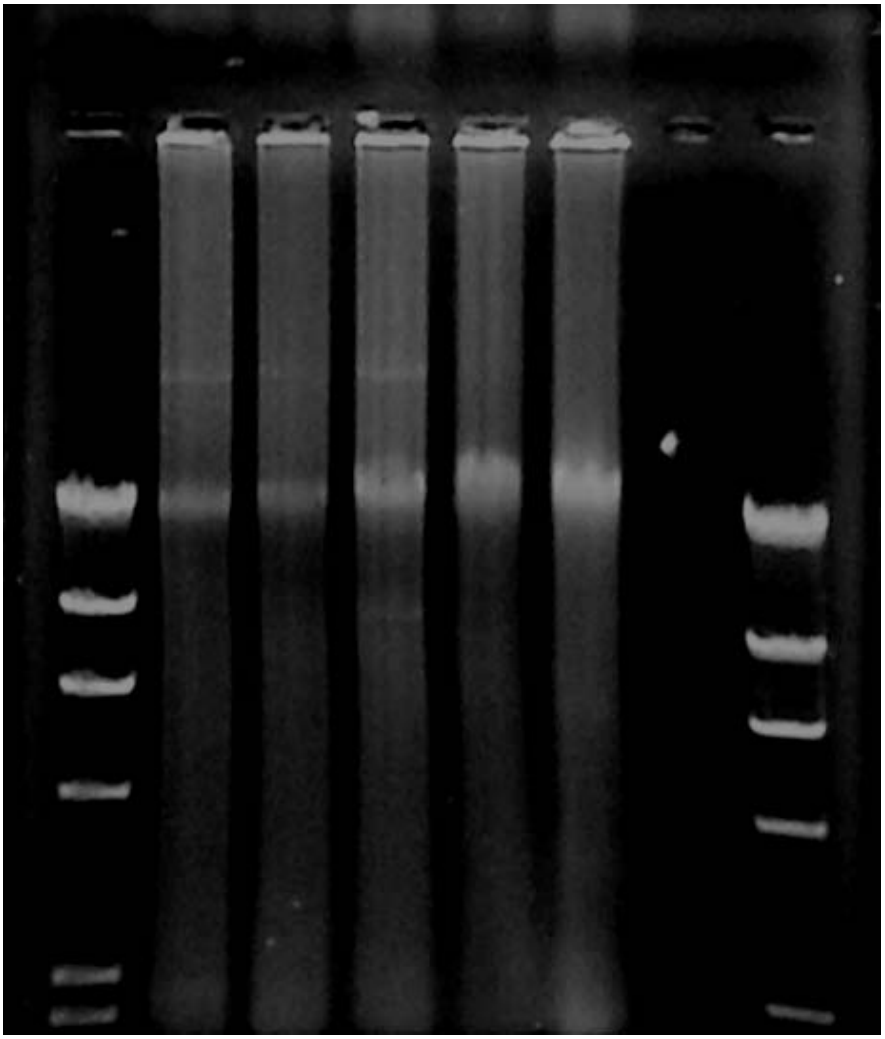

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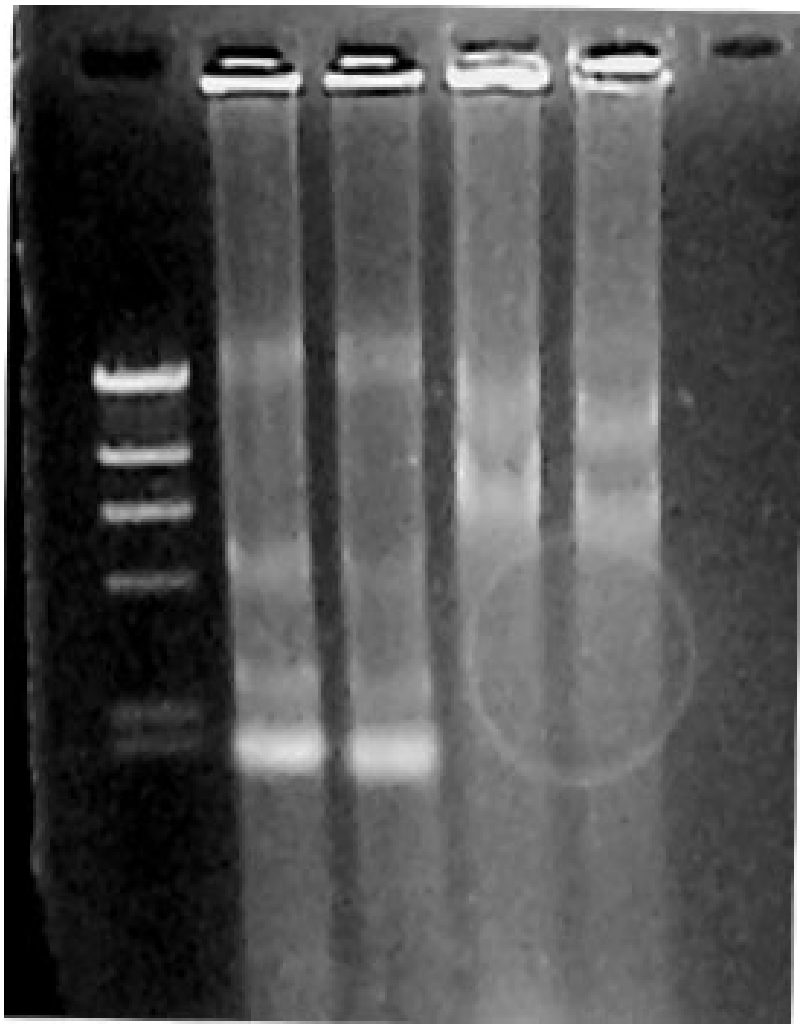
Figure 2.
1. *λ/Hind*III
2. plasmid DNA cut with *Eco*RI, human
3. plasmid DNA cut with *Eco*RI, human
4. plasmid DNA cut with *Eco*RI, chicken
5. plasmid DNA cut with *Eco*RI, chicken



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The investigated Salmonella strains were isolated in specific conditions, almost exclusively in cases of an epidemic or epizootic. The most frequent phage type of 40 *S*. Enteritidis strains were PT 9a (Table 1). Analyzing plasmid profile of human strains compared to chicken ones did not reveal significant differences. Of all strains investigated, 6 did not have any plasmid, 31 strains had only one of 25 kpb, and 3 had an additional plasmid of 2.5 kpb. Figure 1 show the plasmids in a 5 *S.* Enteritidis PT 9a strains. Restriction pattern of *S.* Enteritidis PT 9a either human or chicken origin digested with *Eco*RI showed only differences according to origin (Figure 2).
Phage typing or plasmid analysis could not be used as sole tool for epidemiological study, probably because of phage or plasmid instability (Chart *et al.*, 1989; Threlfall *et al.*, 1993). Also plasmid can be loosed during manipulation in the laboratory. Plasmid restriction analysis shows differences between strains of different origin, but for more precise detection of restriction pattern, analysis of total DNA has to be used. The sensitivity of this method is increased proportionally to the number of enzymes used in the analysis. The investigation performed is only preliminary.

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EFFECT OF HOUSING SYSTEMS ON THE BACTERIAL FLORA IN THE AIR AND ON EGG SHELLS

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Keywords : housing systems, laying hens, environmental air, egg shell contamination

Abstract

The effects of two layer housing systems on the total aerobic mesophilic flora on air and egg shells were investigated during a production cycle.
The following factors were tested : two housing types (aviaries *versus* 3-tier batteries), two husbandry techniques (beak trimming or not).
In our conditions, the air in batteries had a significantly lower bacteria concentration ($3.02\ 10^4$ cfu/m^3) than in aviaries (respectively $5.13\ 10^6$ cfu/m^3 and $2.14\ 10^6$ cfu/m^3 where beak-trimmed and non-beak-trimmed hens were housed). The total aerobic mesophilic flora of egg shells was respectively $1.45\ 10^4$ cfu/egg laid in batteries, $1.78\ 10^5$ cfu/egg laid by beak-trimmed or non-beak-trimmed hens in aviaries.

Résumé

L'incidence de deux systèmes d'élevage de pondeuses d'œufs de consommation sur l'évolution de la qualité bactériologique de l'air ambiant et des coquilles des œufs de consommation a été étudiée au cours d'une année de ponte.
Les facteurs suivants ont été testés : deux systèmes d'élevage, à savoir les volières *versus* les batteries à 3 étages, l'épointage ou non des pondeuses.
Dans nos conditions expérimentales, les résultats obtenus mettent en évidence un effet très significatif du type d'élevage. La densité bactérienne moyenne de l'air s'élève à $3.02\ 10^4$ bactéries/m^3 d'air pour les batteries, à $5.13\ 10^6$ et $2.14\ 10^6$ bactéries/m^3 d'air pour les volières hébergeant respectivement des pondeuses ayant ou non subi un épointage du bec à l'âge de 9 jours. La flore aérobie mésophile des coquilles d'oeufs est de $1.45\ 10^4$ bactéries/oeuf pour les batteries, de $1.78\ 10^5$ bactéries/oeuf pour les volières, que les poules aient subi ou non un épointage du bec.

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Introduction

At laying, the contents of the egg laid by a healthy hen is usually sterile. As for the shell surface, it rapidly becomes contaminated by environmental microorganisms present in droppings, dusts, equipment, etc… (Mayes and Takebalki, 1983 ; Protais *et al*., 1989), with some bacteria being able to pass through the shell and contaminate the internal contents. Many factors can favor or improve this bacterial load (Protais *et al*., 1989 ; Kuo *et al*., 1997 ; Chavez *et al*., 2002).
The purpose of this study is to show possible differences in the bacterial contamination of ambient air and commercial egg shells depending on two layer housing systems, namely batteries *versus* aviaries with beak-trimming *versus* non-beak-trimming of laying hens.

Materials and methods

Laying hens :
From 1 day to 20 weeks of age (W20), 10 640 pullets of the commercial crossbreed ISA Brown were raised in two groups, one on the floor (group 1) and the other in an aviary (group 2). At 9 days of age, half of the pullets in each group had their beak trimmed (group 1BT *versus* group 1NBT ; group 2BT *versus* group 2NBT).
At W20, pullets from groups 1 and 2 were respectively transferred into batteries with 5 hens per unequipped cage and into aviaries equipped with nests, perches and wire floor (of Natura type). Birds from groups 1BT and 1NBT were alternately allocated to the 4 battery rows, which prevented any distinction in the environment of each group. The aviary was divided into 2 parts (2x2660) separated by a grid, measuring 178 m^2 with 3 levels of duckboards and occupied by groups 2BT and 2NBT respectively, thereby individualized. Characteristics of batteries and aviaries are presented in Table 1.
During the laying period, feed was distributed 5 and 6 times daily in batteries and aviaries respectively, to prevent waste resulting from a too large distribution of feed.
Average light intensity was 16 lux in aviaries (from 2 to 26 lux depending on tiers) and 9 lux in cages (from 5 to 12 lux depending on tiers).
The evolution of temperatures in aviaries and batteries during the laying period was very comparable ; only hygrometry was higher by 4 to 5 % in aviaries than in batteries.
Air samples :
The ambient air quality was assessed on the basis of 2 parameters : dust concentration and mesophilic aerobic flora.
Dust concentration was calculated using Renault's method (1997). Dusts, which are borne in the houses' ambient air, were collected on a 0.7 µm-filter through which

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approximately 3 m^3 of air were passed in 2.5 hours. Measures were expressed in g/m^3 and were taken at 27, 39 and 49 weeks of age.
Three other air samples per measuring point were taken, always in the morning and outside periods of agitation for animals (Renault, 1997). Sampling was carried out in corridors and battery rows at two precise points for group 1, and at 2 points per aviary for groups 2BT and 2NBT. Given the animal distribution in batteries, group 1, group 2BT and group 2NBT factors were studied. Samples were collected at approximately 1.50 m from the ground. A sterile filter-holder with a 25 mm-opening and a 0.2 µm-filter was adapted to the tip of a Dräger pump (Renault and Aubert, 1997 ; Protais *et al*., 2003). 400 to 1000 ml of air, corresponding to four to ten pumpings, were collected in batteries and aviaries respectively, at each measuring point, depending on the amount of dust in houses.
The various measuring points were set for the whole course of the experiment.
Samples were collected prior to arrival of animals, at 27, 39, 49 and 60 weeks of age.
Filters were placed in culture in buffered peptone water (BPW) (AES, ref. AEB 140302), with 5 ml BPW per filter ; and dilutions were subsequently done when needed. The mesophilic aerobic flora (MAF) was studied after seeding 100 µl of the BPW solutions and dilution–1 on a PCA agar (AES, ref. AEB 150702) using a rake or a spiral plater, and incubating them at 30°C for 48 hours.
Bacteriological results, which were turned into log CFU/m^3 in order to obtain normal laws and treated as continuous variables, were used for comparisons of the means performed using an analysis of variance.

Eggs :

At 27, 39, 49 and 60 weeks of age, an average of 10 batches of 3 eggs each were collected randomly per husbandry technique from a range of eggs for human consumption and representative of a 2-day laying period. Eggs laid on the floor or on wire floors were subjected to the same analyses. The microbial quality of eggs coming from the batches mentioned above was assessed by counting the mesophilic aerobic flora on a PCA agar (AES, ref. AEB 150702) incubated at 30°C for 48 hours.
The flora present on shells was collected according to the method described by Protais *et al*. (1989, 2003). Three commercial eggs were placed in a bag containing 200 ml buffered peptone water (AES ; ref. : AEB 140302), where shells were delicately rubbed for 2 minutes. From this primary solution made of the homogenized mixture, a 1/10 dilution was done in a tryptone salt medium. 50 to 100 µl were seeded on a PCA agar using a spiral plater.
The bacterial count was brought to the egg and subsequently to the egg's surface according to Bonnet and Mongin's formula (1965).
Bacteriological results were transformed into log CFU/m^2 so as to obtain normal laws, treated as continuous variables and used for a comparison of the means performed using an analysis of variance.

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Results

Hygienic quality of the air :

Prior to arrival of laying hens

In batteries and aviaries, the dust concentration was nil and the bacterial density was under the detection limit.

During the laying period

Results are shown in Table 2 and Figure 1.

The evolution of dust concentration in the ambient air seems to be greater in aviaries than in batteries.

The comparison of results shows a significant difference in the air quality between aviary groups BT and NBT ($F = 6.53$; $P = 0.02$) while no difference is observed between the times of analysis ($F = 1.89$; $P = 0.18$) nor any interaction between these factors ($F = 1.97$; $P = 0.17$).

The average mesophilic aerobic flora of the air reaches 6.88 log CFU/m^3 of air in the aviary housing the beak-trimmed birds, and 6.26 log CFU/m^3 of air in the aviary housing the non-beak-trimmed birds.

The heavier load of airborne bacteria in aviary BT may result from the type of activities of this group, which spent more time in a nutrition behavior (eating and drinking), although its feed and water consumption was lower than that of aviary group NBT.

As this discrepancy between aviaries prevented their pooling, the same comparison was done between the contaminations in all 3 "batteries", "aviary BT" and "aviary NBT" groups, which revealed a significant incidence of housing systems ($F = 32.4$; $P < 0.0005$), but no "time of analysis" effect ($F < 1$) and no interaction ($F = 1.87$; $P = 0.12$)

The average mesophilic aerobic flora of the air reaches 4.21 log CFU/m^3 of air in batteries. Values obtained in batteries are very close to those obtained by Zucker *et al*. (2000) under very similar conditions but using a different sampling system, ant to those obtained by Renault and Aubert (1997).

This difference between housing systems could partially be attributed to the production of drier droppings in aviaries and consequently, to a different hygrometry. It seems that this factor of 100 cannot be equally explained by the notion of available volume per animal or of density per square meter. Obviously, these notions do not permit the comparison of the housing systems. In fact, our results brought to the available volume of air per laying hen (0.3 m^3 per hen in batteries and in aviaries) or to the density per m^2 of available surface per bird (9 and 17.2 per m^2 respectively) are opposed to the results of Sauter *et al*. (1981), who showed evidence of a link between the number of bacteria in the air and the available volume per bird (0.42 *versus* 0.84 m^3) in layer housing systems between 25 and 36 weeks. However, our results confirm those of Renault

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(1997), who observed little variation of the MAF depending on the density in turkey housing systems.
No "age of laying hens" effect was observed whereas Madelin and Wathes (1989) noted an age-related increase in the bacterial load in systems housing broilers bred on litters.

Microbiological quality of the egg shell :

Detailed results are shown in Table 3.
The statistical analysis performed on mesophilic aerobic flora counts shows no significant effect of beak-trimming or non-beak-trimming of laying hens ($F<1$). On the contrary, significant differences are noted for the following factors : "housing systems" ($F : 751.02$; $P<0.0005$, "age of laying hens" ($F : 10.27$; $P<0.0005$) and the interaction "housing systems x age of laying hens" ($F : 9.80$; $P<0.005$).
It is reminded that the eggs used were for human consumption, for which eggs with cracked or dirty shells, etc…, are not selected.
The shell contamination seems quite stable during the laying year, save at the end of the period (60 weeks), when it seems to be slightly decreased, particularly in aviaries.
Commercial eggs laid by laying hens raised in aviaries show a shell contamination much higher than that of eggs laid in batteries (4.16 ± 0.26 *versus* 5.25 ± 0.30 log CFU/egg). Brought to the egg's surface, the shell contamination reaches 2.28 ± 0.26 log CFU/cm^2 and 3.38 ± 0.31 log CFU/cm^2 in groups 1 (batteries) and 2 (aviaries), respectively. In that case, the same significant differences can be observed for the same factors. Values obtained in batteries are comparable to those published by Protais *et al* (1989) and Chavez *et al*. (2002) for eggs in shells for human consumption.
Contamination of shells of eggs produced in aviaries exceeds that of eggs produced in batteries by at least one logarithm. This increase seems to be linked to the higher level of airborne particles and bacteria in aviaries than in batteries.
Eggs laid on the floor or on wire floors are much dirtier and showed a greater contamination than that of visually clean eggs. The mean value was 6.86 ± 0.70 log CFU/egg (4.99 ± 0.70 log CFU/cm^2), with respectively 6.91 ± 0.73 log CFU/egg (5.02 ± 0.73 log CFU/cm^2) and 6.70 ± 0.56 log CFU/egg (4.83 ± 0.55 log CFU/cm^2) for shells of eggs laid on the floor or on wire floors in aviaries. These values are in accordance with those observed by Protais *et al.* (1989).

Conclusion

Under our experimental conditions, during this laying year, the air contamination by the mesophilic aerobic flora reached an average of $1{,}7\ 10^4$ bacteria/m^3 of air in batteries, $5{,}9\ 10^6$ and $2{,}75\ 10^6$ bacteria/m^3 of air in aviaries housing beak-trimmed and non-beak-trimmed laying hens, respectively. The mesophilic aerobic flora on shells of eggs produced by laying hens in batteries or aviaries was on average $1.45\ 10^4$ bacteria/egg

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(or 195 bacteria/cm^2) and 1.78 10^5 bacteria/egg (or 2400 bacteria/cm^2) respectively, whether hens were beak-trimmed or not.
In aviaries, the air, which is more loaded in dusts, contains an increased number of airborne bacteria and contaminates egg shells, animals and even the breeder.

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Table 1: Characteristics of the two layer housing systems

Characteristics		4 batteries	2 aviaries
Available surface per animal (cm^2)		582	1110
Density (birds/m^2 of availble surface)		17.2	9
Number of pipettes per animal		0.2	0.1
Length of feeding trough per animal (cm)		12	9
Special equipement	Litter (cm^2/animal)	absence	410
	Nest (cm^2/animal)	absence	256
	Perches (cm/animal)	absence	14.7

Table 2 : Weight of dusts (g/m^3 of air)

	Age of laying hens (weeks)		
	27	39	49
Batteries	$9.9\ 10^{-4}$	$1.6\ 10^{-3}$	$1.4\ 10^{-3}$
Aviaries 1	$7.8\ 10^{-3}$	$1.4\ 10^{-2}$	$8.7\ 10^{-3}$
Aviaries 2	$7.7\ 10^{-3}$	$2.4\ 10^{-2}$	$1.6\ 10^{-2}$

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Figure 1 : Evolution of the mesophilic flora in the house

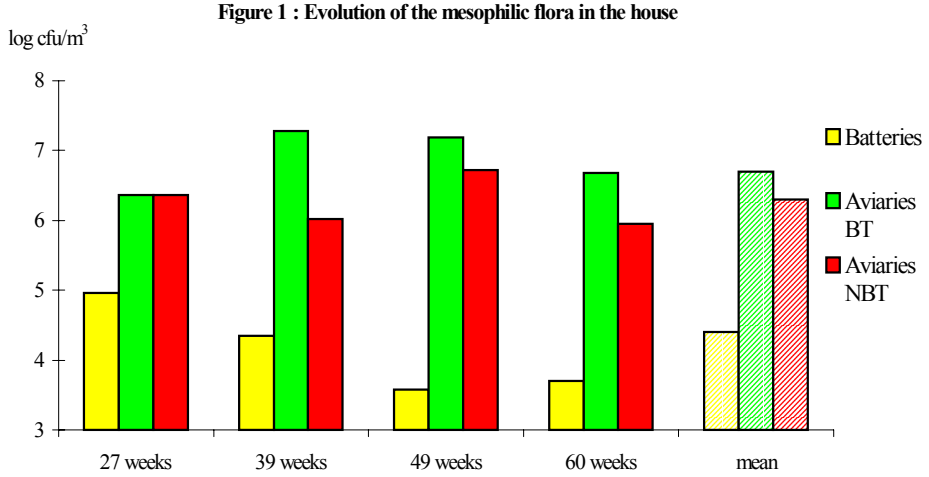

Table 3 : Evolution of the mesophilic aerobic flora present on egg shells (log CFU/egg)

	Age of laying hens (weeks)				
Groups	27	39	49	60	Mean
Group 1BT (batteries)	4.01 ±0.25	4.11 ±0.35	4.17 ±0.30	4.12 ±0.24	4.10 ±0.28
Group 1NBT (batteries)	4.19 ±0.29	4.33 ±0.18	4.19 ±0.17	4.17 ±0.28	4.21 ±0.24
Group 2BT (aviaries)	5.43 ±0.19	5.57 ±0.22	5.29 ±0.19	4.91 ±0.21	5.30 ±0.31
Group 2NBT (aviaries)	5.45 ±0.32	5.26 ±0.12	5.16 ±0.24	4.89 ±0.21	5.19 ±0.29
Group 1 (batteries)	4.10 ±0.28	4.23 ±0.27	4.18 ±0.23	4.14 ±0.25	4.16 ±0.26
Group 2 (aviaries)	5.44 ±0.24	5.41 ±0.24	5.22 ±0.22	4.90 ±0.20	5.25 ±0.30

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SOME FACTORS INFLUENCING THE MICROBIOLOGICAL QUALITY OF FRESH AND PASTEURIZED WHOLE EGGS

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Keywords: fresh and pasteurized whole eggs, microbiological quality, profiles of contamination

Abstract

Industrialists of eggs processing plants have been having reflection for many years on their professional practices and the amelioration they could bring to improve the quality of egg products (fresh or pasteurized). The first step was to better know the factors linked to the alteration and potentially pathogen floras of the whole eggs. In this context three companies, seven microbiological analyses laboratories and statisticians conducted a study in "Bretagne" and "Pays de Loire". Underscoring of significant factors linked to the contamination of fresh and pasteurized whole eggs, such as season, use-by date, gives the manufacturers important elements to nourish their reflection.

Résumé

La recherche d'une qualité optimale des ovoproduits crus et pasteurisés conduit depuis de nombreuses années les professionnels à réfléchir sur leurs pratiques et sur les améliorations possibles. Celle ci passe par une première phase de meilleure connaissance des facteurs liés à la flore d'altération et potentiellement pathogène de ces produits. Dans cette optique 3 entreprises, 7 laboratoires d'analyses microbiologiques et des statisticiens ont conduit une étude en Bretagne et Pays de Loire. La mise en évidence de facteurs liés à la contamination des ovoproduits, tels que la saison, la destination du produit, donnent aux professionnels des éléments importants pour alimenter leur réflexion.

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Introduction

Nowadays microbiological quality of eggs and egg products is a preoccupation for European authorities. The search of optimal quality has been one of the main aims of industrial people for many years. They also had reflection about their professional practices and the quality of their manufactured products.
In this context, three egg processing plants, seven microbiological analyses laboratories and statisticians conducted a study financed by French administrative regions of "Bretagne" and "Pays de la Loire".
The goal was to identify the alteration and potentially pathogen flora in fresh and pasteurized whole eggs and to find out the effects that could be linked (season, professional practices…).

Materials and methods

Three companies participated in this study. Samples were taken at three seasons : winter (February and March 2000), summer (June and July 2001) and autumn (October to December 2001).
The way of sampling was defined with an experimental design comprising five factors having each one two modalities : time in the day (morning or evening), poultry age (4-6 months or 9-12 months), duration of eggs storage (less than three days or 8-10 days), duration of whole eggs storage to a low temperature (2 or 5 hours), destination of the product in terms of use-by date (14 or 49 days).
Criteria and organisation of this design were defined according to statistical hypothesis, production and laboratories constraints.
For each season and each company, a 16 lines design was fixed (data base has 144 lines -3 seasons * 3 companies * 16 lines). A questionnaire, with fixed criteria and some questions about other parameters or production mode, had to be filled in by industrial people. For each line, three analyses were done : one on the fresh whole eggs (before pasteurization), one on the pasteurized one and one on this same pasteurized product at the use-by date after storage at 2°C.
The multidimensional analysis on the three periods is here presented. This one was realised in three steps : one for the results on fresh whole eggs, one for the results on pasteurized ones and one on the use-by date products. Data about microbiological results were centred by company, to avoid the effect "contamination level" particular for each of them. Principal Component Analyses (PCA) were produced followed by analysis of variance (ANOVA) on principal components. The signification of the modalities is associated to a p-value < 0.01.

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Continuous and active variables retained for the PCA were : *Bacillus*, *Enterococcus*, Enterobacteriaceae, *E. Coli*, yeast, mould, lactic flora, *Pseudomonas*, *Streptococcus*, psychrotrophic flora, *Brochothrix*.
Continuous and illustrative variables were : Mesophilic Aerobic Flora, *Bacillus Cereus*, lysozyme and protein.
Nominal and illustrative variables are the factors from the experimental design and from the questionnaire : company, season, poultry age, duration of eggs and fresh whole eggs storage, time in the day for the sample, use-by date, selection of eggs or not, grading of eggs or not. Interactions between these variables are also introduced in this analysis.

Results

Analysis on fresh whole eggs (before pasteurization)

Individuals are displayed on figure 1, active and illustrative continuous variables on figure 2. The percentage of inertia on the first two axes is 50,56%. A size effect appears, the main variability effect opposes the high contaminated samples (on the left of the charts) to the least contaminated ones. On the first two axes, three variables groups are distinguished :

- group 1 : *E. Coli*, *Brochothrix*, *Enterococcus*, Enterobacteriaceae
- group 2 : psychrotrophic flora, *Pseudomonas*, *Streptococcus*, mould, *Bacillus*
- group 3 : lactic flora, yeast

The Mesophilic Aerobic Flora was counted by two laboratories. The results of the first one (MAF1) seem to be associated to the level of *Streptococcus* whereas the results of the second one (MAF2) seem to be associated to the psychrotrophic flora.
Two factors seem to be linked to the contamination level of fresh whole eggs : season and use-by date.
The contamination level is higher in summer, and lower in winter. The season effect is emphasised in the third company. We can notice that the second company is also particular. The contamination level in winter is significantly higher than the results we could expect. A possible explanation is that the temperature of the winter samples (for this company) at their arrival in laboratories was really higher than the recommended temperature (nearly 10°C *versus* 4°C).
The use-by date effect is essentially linked to company 2. A use-by date of 14 days is associated to the samples highly contaminated in lactic flora and yeast when a use-by date of 49 days is opposed to them. Let's remember that no pasteurisation was done at this step (fresh products). The use-by date is in fact the type of products in terms of destination (14 or 49 days of maximal conservation).
The analysis of variance on principal components confirmed the effects previously listed (p-value less than 0.01) : summer, winter, interaction company 2 * season.

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Figure 1 : results of CPA on fresh whole eggs, representation of individuals (axes 1 and 2)

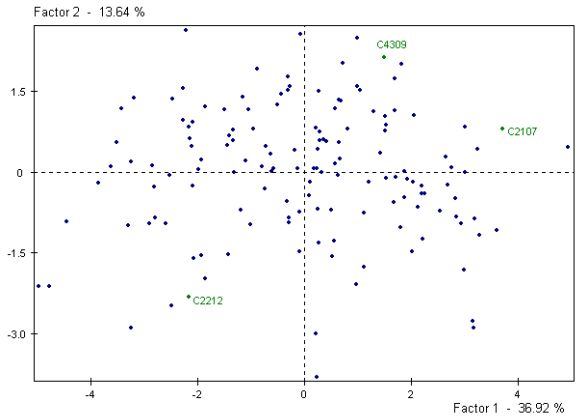
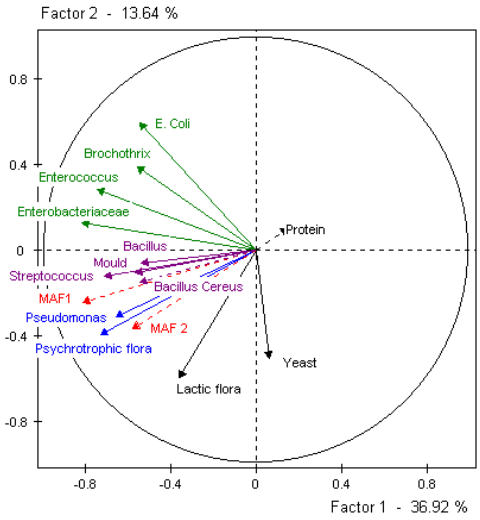


Figure 2 : results of CPA on fresh whole eggs, representation of continuous variables (active and illustrative – stippled drawing)



Three individuals were selected on figure 1. We can verify our hypothesis by their characteristics. Let's remember that the mean of each variable is 0 (data were centred by company). The C2212 (on the left) has positive values for all variables except *E. Coli* (- 0,425). It is also a sample with a higher contamination comparatively to the other.

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The C 2107 (on the right) has negative values for all variables except *Bacillus* (1,080). It is also a sample less contaminated than the other. The C4309 (on the top) has negative values, but slightly higher than the values for C2107. Only one value is positive, for *E. Coli* (0,096). That is in accordance with the results showed on figure 2.

Analysis on pasteurized whole eggs

Individuals are displayed on figure 3, active and illustrative continuous variables on the figure 4. The percentage of inertia on the first two axes is 36,80%. The pattern is here clearly different than for fresh whole eggs. It appears on the individuals figure (3), and it is confirmed on the variables figure (4). The first axis opposes the samples with a high level of *Enterococcus* and *Streptococcus* to the least contaminated by these two floras. The second axis opposes the samples with a high level of *Pseudomonas* and psychrotrophic flora to the least contaminated by these two floras.

Season and use-by date are the main effects associated to the contamination variations between samples of pasteurized whole eggs. So, there are few *Enterococcus* and *Streptococcus* in autumn comparatively to the two other seasons. Concerning *Pseudomonas* and psychrotrophic flora, contamination is lower in winter and higher in summer. As in fresh products, the season effect is highly emphasised in the third company and the particularity of the second one is confirmed (higher contamination level in winter than the results we could expect).

The use-by date effect is also essentially linked to the second and the first companies. Each of them has its own pasteurization scale (a time / temperature couple), according to the use-by date. Differences between the scales can explain the interactions of the company and the use-by date variables. A use-by date of 14 days is associated to the samples highly contaminated in *Enterococcus* and *Streptococcus* excepted for the first company where the 49 days use-by date is associated to these floras.

Figure 3 : results of CPA on pasteurized whole eggs, representation of individuals (axes 1 and 2)

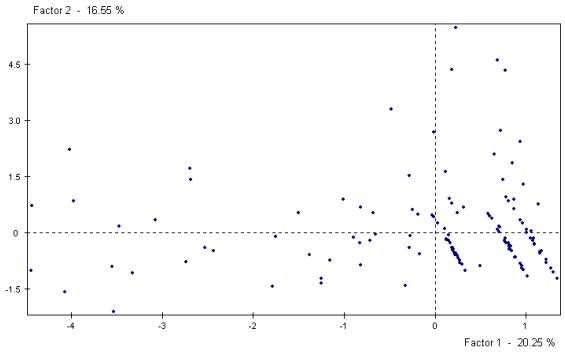

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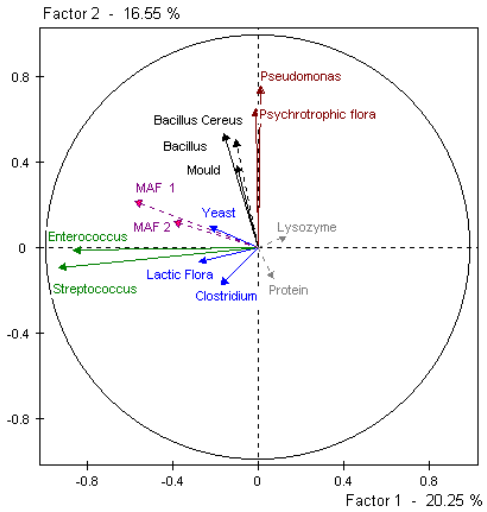
Figure 4 : results of CPA on pasteurized whole eggs, representation of continuous variables (active and illustrative – stippled drawing)



Analysis on use-by date whole eggs

The percentage of inertia on the first two axes is 30,88%.

The pattern of individuals is near to the one on pasteurized products but on different axes. The first one opposes the samples with a high level of *Pseudomonas* and psychrotrophic flora to the least contaminated by these two floras. The second axis opposes the samples with a high level of *Enterococcus* and *Streptococcus* to the least contaminated by these two floras.

As it was previously mentioned, season and use-by date are the main effects associated to the contamination variations. So, there are few *Enterococcus* and *Streptococcus* in autumn and higher levels are in winter. The level of *Pseudomonas* and psychrotrophic flora is slightly higher in summer comparatively to the two other seasons.

The season effect is also emphasised in the third company but differently than in the two previous analyses. We can also notice that the contamination level for the winter samples is “abnormally” higher in this company 3 comparatively to the two others.

The use-by date effect is here slightly different than previously. The samples highly contaminated with *Pseudomonas* and psychrotrophic flora are associated to a 14 days use-by date for the three companies. We can also notice that this effect is emphasised in the second one. The use-by date effect is significant for *Streptococcus* and *Enterococcus* only if it is associated to the season. The contamination level of the summer samples intended to a 14 days use-by date is higher than the one for summer samples intended to a 49 days use-by date.

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Conclusion / Discussion

This study required a close collaboration between industrialists, financial people, microbiological analyses laboratories, statisticians. The conception of the experimental design was the first important step. This one was established on statistical attributes then discussed and validated by other partners.
The analysis of the microbiological results enabled to show the varied contamination of the samples in terms of pattern, if we talk about fresh whole eggs (size effect), pasteurized or use-by date ones (pattern according to *Pseudomonas* and psychrotrophic flora, *Streptococcus* and *Enterococcus*).
The season is the main effect for all analyses (fresh, pasteurized and use-by date whole eggs). The interaction with companies is significant. Several explanations are possible : high temperature of some samples at their arrival in laboratories, contamination of the environment different between the three companies, various contamination levels of eggs according to the poultry houses…
Another major factor is the use-by date effect. This one is more significant in one of the three companies, even in fresh, pasteurized or use-by date whole eggs. For the last two ones, it can be explained by the pasteurization scale of this company. For fresh products, a sort of eggs even involuntary according to the type of the products (short or long conservation) is not to be excluded.
Significantly factors could be detected on all samples thanks to a conjoined analysis of the results, by Multiple Factorial Analysis (only one analysis for fresh, pasteurized and use-by date results).

Acknowledgements :

This study was financed by the PAO (Pôle Agronomique Ouest – Bretagne et Pays de Loire). The author wants to thank J. Pagès (INSFA) for his advice and help during the data processing, the three egg processing plants, ABCD (Ploërmel-56), Société Geslin (Chauche-85), 3 Vallées (Ambrières les Vallées-53), and the seven microbiological laboratories, ADRIA - V. Huchet - (Quimper-29), AFSSA - J. Protais, G. Salvat, K Rivoal, E. Boscher, S. Queguiner - (Ploufragan-22), ASEPT - F. Bourion - (Laval-53), ENSAR - M. Gauthier, F. Baron - (Rennes-35), ENVN - M. Federighi - (Nantes-44), ESMISAB - D. Thouvenot - (Brest-29), NUTRINOV - P. Lorthioir, V. Gourvès - (Rennes-35), who made this work possible.
Many special thanks to Jérôme Pagès and Jocelyne Protais for their precious help during the writing of this article.

CONTENTS

EGG QUALITY AND MYCOTOXIN RESIDUES OF LAYING HENS FED A DIET CONTAINING AFLATOXIN B1 AND ESTERIFIED GLUCOMANNANS

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Keywords: Aflatoxin B1, esterified glucomannan, residues, egg quality, laying hen.

Abstract

Seventy two laying hens were allocated to four groups administered different diets over a 4 week period (0-0: control; EGM-0: diet supplemented with 0.11% esterified glucomannans- EGM; 0-AF: diet supplemented with 1.8 ppm Aflatoxin B1-AF; EGM-AF: diet supplemented with 0.11% EGM *plus* 1.8 ppm AFB1) to evaluate the effect of AFB1 or/and EGM on egg quality and the ability of EGM to interact with an oral administration of AFB1. Egg deposition and weight decreased in the group fed diet EGM-0 *versus* group 0-0. AFB1 also influenced colour parameters and liver weight. Eggs and livers showed no residues of aflatoxin, while the *excreta* resulted positive for the mycotoxin and the highest amount (P>0.05) of the mycotoxin was observed in *excreta* of the animals administered the diet containing AFB1 *plus* EGM.

Résumé

72 poules pondeuses ont été subdivisées en 4 groupes et alimentées différemment pendant 4 semaines (0-0: contrôle; EGM-0: alimentation additionnée de 0,11% de EGM- glucomannanes estérifiés; 0-AF: alimentation contenant 1,8 ppm d'aflatoxine B1; EGM-AF: alimentation contenant 0,11% d'EGM et 1,8 ppm d'AFB1) pour mesurer les effets de l'AFB1 et de l'EGM sur la qualité des œufs et la capacité d'interaction des EGM avec administration orale d'AFB1. La ponte des œufs et le poids ont diminué dans le groupe à alimentation EGM-0 par rapport au groupe 0-0. L'aflatoxine a conditionné les paramètres de couleur et de poids du foie. Œufs et foies ne présentaient aucun résidu d'aflatoxine, tandis que les excréments se sont avérés positifs à la mycotoxine; les quantités les plus élevées de mycotoxine (P>0,05) ont été enregistrées chez les animaux ayant reçu le régime AFB1 plus EGM.

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Introduction

Cereals are a major mycotoxin vector, as 25-30% of them are contaminated with such substances. The most dangerous of these toxins include aflatoxins (AFs) produced by *Aspergillus flavus* and *Aspergillus parasiticus.* AFs are immunosuppressants, hepatotoxics, hepatocarcinogens, teratogens and mutagens. In layers the effects of exposure to AFs include a dose-dependent decrease in egg production and increased susceptibility to salmonellosis, candidiosis and coccidiosis (Celik et al., 1996). In the case of laying hens, some studies have shown that AFs and their metabolites, aflatoxicol in particular, can also be carried over to eggs in a ratio of mycotoxin content in feed and in egg ranging 5,000: to 66,200:1 and as high as 125,00: 1, whereas in other experimental trials measurable residual AFB1 and/or its metabolites were not found in eggs (Wolzak et al., 1985; Bertuzzi et al., 2001; Zaghini et al., 2001; Rizzi et al., 2003a, b). These highly contrasting results may be ascribable to the supply of naturally contaminated feeds or diets containing different aflatoxin fractions (Oliveira et al., 2000). Various treatments and dietary strategies have been applied in an attempt to reduce aflatoxin levels in contaminated commodities and their oral bioavailability. A *Saccharomyces cerevisiae* cell wall derived binder, an esterified glucomannan (EGM- Mycosorb, Alltech Inc.) displays a high *in vitro* capacity (95%) to bind AFB1 (Devegowda *et al*., 1998). In this experimental trial the *in vivo* effectiveness of EGM in reducing AFB1 toxicity in laying hens and the effects of these two compounds on egg quality and chemical parameters were assessed.

Material and Methods

Seventy -two Warren hens were divided into four body weight and egg production matched groups of 18 animals each. At the beginning and at the end of the trial, all the birds were weighed to determine weight gain. The experimental design provided for 4 dietary treatments: 0-0: control, complete diet; 0-EGM: diet supplemented with 0.11 % EGM; AF-0: diet supplemented with 1.8 ppm AFB1; AF-EGM: diet supplemented with 1.8 ppm AFB1 and 0.11% EGM. The 0-EGM diet was included to provide information on the effects of the adsorbent when fed alone. AFB1 (produced by *Aspergillus flavus*, code A6636, purity > 99%) was purchased from Sigma Aldrich S.r.l. (Milan, Italy). EGM (Mycosorb) was supplied by Alltech, Inc. (Dunboyne, Co. Meath, Ireland). Before feeding, the basal diet was tested by means a HPLC method to ensure that it contained no residual mycotoxins. Throughout the study (4 weeks) feed and water were provided *ad libitum*. Feed consumption and egg production were determined weekly and daily respectively. Some physical and qualitative parameters

were determined in eggs laid over a three- day period at 0 and 4 weeks from the beginning of the treatment: egg weight, percentage of yolk, albumen and shell, Haugh index, yolk colour, measured using a Minolta Chromometer Reflectance CR 200/08 (Barletta Apparecchi Scientifici S.r.l., Milan, Italy) with D65 Illuminant, and shell thickness. The colour number (CN) was determined according to the method proposed by Scholtyssek (1995). The chemical composition of yolk was determined, using AOAC (1995) methods 950.46, 984.13, 925.32 and 942.05 to assess moisture and, after freeze drying, crude protein, ether extract and ash respectively. At the end of the trial, all the animals were euthanized and livers were weighed. The presence of AFB1 was determined by the same analytical method in eggs collected at 0 and at 4 weeks, in *excreta*, and in livers, using the Veratox Quantitative Aflatoxin Test (Neogen®, 620 Lasher Place, Lancing MI 48912). The detection limit of immunoenzymatic test was 1 ppb. The data were subjected to analysis of variance and the means were compared using Student's t test.

Results and discussion

During the experimental period, all the birds undergoing treatment were healthy, and no pathological macroscopical lesions were apparent in the oral cavity, proventriculus, small or large intestine, spleen, liver, heart, or kidney. Livers of groups contaminated by AFB1 showed a higher weight ($P>0.05$) than organs of the other groups. No significant differences between the four groups were found with regard to the qualitative characteristics of eggs collected before the beginning of the trial, hence the data are not shown. There was no apparent effect of the mycotoxin and EGM on weight gain and feed intake, although an insignificant decrease in these parameters ($P >0.05$) was noted in the laying hens fed diets AF-0 and 0-EGM. Egg laying in hens receiving the diets containing AFB1 or 0.11% EGM was negatively influenced ($P <0.01$) by feed intake (table 1). Groups fed the diets contaminated by AFB1, with or without a supplement of EGM, showed the same zootechnical performances as the control group. In previous experimental trials (Rizzi et al., 1999, 2001; Simioli et al., 2001) no significant effect of the mycotoxin or EGM was observed on egg laying. It appears that egg production is adversely affected only when the hens are exposed to higher concentrations of AFB1. Iqbal et al. (1983) and Sudhakar (1990) observed such an effect with diets containing AFB1 (or Aflatoxin B1 equivalent) levels above 600 µg/kg feed. After 28 days, egg weight was negatively influenced by the addition of the EGM to the diet (table 2). This effect was also observed when clinoptilolite or a mannoligosaccharide was used as a mycotoxin binder (Rizzi et al., 2003a, b). Washburn et al. (1984) observed both a reduced egg and yolk weight in groups of hens fed a diet contaminated with 5µg of AFB1 per g of feed, which is an amount higher

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than that used in our trial. Egg components and shell thickness were similar across the four groups. The mycotoxin appeared to influence colour parameters. The variation in colour might be connected to the interference of AFB1 with lipid metabolism (Tung et al., 1972) and also with pigment deposition in yolk, as Genedy et al. (1999) similarly observed. Carotenoids have a dietetic origin, while other lipids are essentially synthesized *de novo* in the liver. The complexity of mycotoxin interactions is demonstrated by the finding that carotenoid absorption was inhibited by aflatoxin to a greater extent in low-fat than in high-fat diets (Smith et al., 1991). Huff et al. (1975) observed a greater yellowness in egg yolks of laying hens fed a diet containing 10 µg/g AFB1. The Haugh index appeared to be improved by supplementing the diets with EGM (0-EGM and AF-EGM), while clinoptilolite, used as a mycotoxin binder, negatively influenced this parameter (Rizzi et al., 2003b). With regard to yolk chemical composition (table 2), no statistically significant differences were observed between the groups. The inclusion of EGM in the diet appeared to increase the protein percentage (P >0.05), as previously found (Rizzi et al., 2003a). The eggs and livers of all the birds did not show residues of AFB1; on the contrary *excreta* resulted positive for the mycotoxin in groups AF-0 and AF-EGM (table 3). Besides the differences between the groups AF-0 and AF-EGM were not statistically significant (P> 005), the highest amount of AFB1 was observed in the *excreta* of the animals administered the diet containing AFB1 *plus* EGM. Probably the low concentration of the aflatoxin administered might explain the absence of statistical significance, in any case our results confirm *in vivo* the ability of EGM to bind AFB1 and to prevent its gastrointestinal absorption.

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Table 1. Feed intake and egg production

	Treatment				
	0-0	0-EGM	AF-0	AF-EGM	SEM
Feed intake (g/d)	141.54	120.95	125.56	140.19	2.23
Egg production (%)	90.23A	64.40B	80.04AB	92.28A	2.87

A, B: P< 0.01

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Table 2. Egg quality and chemical composition of the yolk at the end of the trial

	Treatment				
	0-0	0-EGM	AF-0	AF-EGM	SEM
Egg weight	64.82a	59.60b	62.03ab	62.49ab	0.64
Yolk (%)	26.33	26.52	25.24	25.85	0.21
Albumen (%)	59.07	58.19	60.07	59.65	0.10
Shell (%)	10.03	10.69	10.60	10.64	0.48
Shell thickness (mm)	0.33	0.34	0.34	0.34	0.003
Yolk Colour number	18.02b	18.43ab	18.95a	18.65a	0.26
Haugh index	85.56B	90.08A	85.34B	90.18A	0.71
DM*	51.95	58.99	51.90	51.25	0.97
CP* (% DM)	28.05	30.09	33.79	31.80	0.24
EE* (% DM)	60.51	59.37	59.68	60.11	0.42
Ash (% DM)	2.98	2.89	3.18	3.05	0.02

*DM = dry matter; CP = crude protein; EE =ether extract;
a, b: P< 0.05; A, B: P< 0.01

Table 3. AFB1 levels in *excreta* at the end of the trial (ppb)

	Treatment	
	AF-0	AF-EGM
AFB1	20.11	27.32
SEM	3.28	3.83

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A STUDY ON THE BACTERIAL CAUSES OF DECREASE HATCHABILITY OF SOME HATCHERY IN IRAN

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Keywords: Chicken, eggs , hatch, bacteria, contamination

Abstract

Dead–in–shell chicken embryos from commercial hatcheries in khuzestan state of Iran were studied for isolation of aerobic bacteria. For this purpose a total of 850 eggs with embryos that failed to hatch at termination of incubation were randomly collected over a period of one year and were cultured. One hundred six (12.5%) of the 850 eggs sampled were found to be contaminated. Bacteria isolated from 106 samples were Enterobacteriaceae (44.6% mainly E.Coli) , Staphylococcus spp. (14.3%), Micrococcus spp. (14.3%) , Proteus mirabilis (8%) , streptococcus spp. (7.1 %), bacillus cereus (6.3%) Salmonella spp. (0.9%) Moraxella spp (0.9%), Nisseria (0.9%) and unidentified gram negative bacteria (2.67%). When the flock was older, incidence of contamination in eggs failling to hatch increased.

Résumé

La flore aérobie des œufs embryonnés morts a été étudiée dans les établissements d'incubation commerciaux dans l'état du Khouzestan de l'Iran. À cette fin, un total de 850 œufs embryonnés morts a été aléatoirement prélevé en fin d'incubation pendant l'année et a été cultivé. Cent six des 850 œufs prélevés étaient contaminés. Les bactéries isolées dans 106 échantillons étaient les entérobactériacées(44.6%), principalement E.Coli, staphylocoques (14.3%), microcoque (14.3%), Proteus mirobilis (8%), streptocoques (7.2%), salmonelles (0.9%) et bactéries gram négatives non identifiées (2.67). La mobilité embryonnaire augmente avec l'âge du troupeau reproducteur.

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Introduction

Microorganisms play an important role in the hatchability of fertile eggs. An assessment of the incidence and type of bacterial contamination occurring in eggs produced and hatched in commercial hatcheries is essential to understanding the role that micro–organisms play in influencing hatchability. Some of the observations made regarding table egg maybe applicable to hatching eggs while others may not. The environment prevailing in an incubated egg containing a developing embryo is clearly different from that of infertile egg and is in a state of constant change.In present study the incidence and type of bacterial contamination occurring in sample of incubated eggs which had failed to hatch was assessed.

Materials and Methods

Eight hundred fifty eggs with embryos that failed to hatch at termination of inubation were randomly selected over a period of one year. All samples were examined , and those with cracks and those from wich embryos pipped the shell but failed to emerge were discarded to minimize of extraneous contamination. The eggs were first washed with disinfectant, and allowed to dry. The shells were further disinfected by mopping with ethyl alcohol. The eggs then opened by making a cirular cut with sterile scissors along the outline of the air space before the contents were aseptically pooled in to a sterile beaker. If embryos were fully developed, only the yolks were harvested. The pooled yolks and embryos were homogenized aseptically. The homogenized samples were cultured to isolate aerobic bacteria by streaking 0.2 ml of the homogenized yolk on blood agar and inoculation selenite broth as well as *mycoplasma* broth (PPLO broth) employing the standard bacteriological methods (5). Only those plates containing more than about 50 colonies were selected for further study. Representatives of each colony type were replated until purity was assured and the isolates were stored on slopes of Nutrient Agar at 4^0C.

Results

One hundred six (12.5%) of the 850 eggs sampled were found to be contaminated. bacteria isolated were 42 *Enterobacteriaceae* (44.6%, mainly E.coli), 9 *Proteus mirabilis* (8%),16 *staphylococcus* spp. (14.3%), 16 Micrococcus spp.(14.3%), 8 *streptococcus* spp. (7.1%) , 7 *Bacillus cereus* (6.3%) , 1 *Salmonella spp.* (0.9%),1

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Moraxella spp. (0.9%) , 1 *Nisseria* spp. (0.9%), 3 unidentified gram negative bacteria (2.7%). When the flock was older , rate of contamination in eggs failing to hatch increased.

Discussions

Results of present study showed that Enterobacteriaceae (44.63%) (mainly E.coli) constitued predominant group of bacterial contamination in dead-in-shell eggs and with increasing the age of parent flock contamination increased. Bruce and Johnson(1978) in a similar work reported Micrococcus spp. and Enterobacteriaceae represented 34.6% and 31.5%, respectively, but orajaka and Mohan (1984) in study on the aerobic bacterial flora in dead– in–shell chicks , recovered E.coli 25% and S. aureus 27.18% of bacterial isolates . It is known that E.coli to cause yolk – sac infection in embryo and early chick and embryonic mortality. Also S. aureus , which inhabitis the skin surface of birds and their litters, has been reported to be capable of gaining access to the yolk – sac through shell penetration and to cause embryonic death. The enzyme/ toxins produced by this organism , which may use up intact yolk as substrate , are considered to be responsible for embroyonic death. Burce and Johnson (1978) found the incidence of contamination with pseudomons spp. and Enterobactriaceae in creased signifi catly with flock age ($P<0.01$) , which was thought to reflect a declining level of environmental hygiene , but in present study pseudomonas spp. was not isolated.

Bruce and johnson (1978) demonstrated that as flock became older the incidence of contamination in eggs failing to hatch increased, because the defense mechanisms of the egg deteriorate with flock age, the environment become more contaminated, the egg – laying apparatus of the hen becoms more contaminated with age and produces more contaminated eggs.

In addition it is considered that a general decline in the hygienic status of the environment during the life of the flock and in particular contamination of nest boxes is likely to play an important role.

Mycoplama spp. was not isolated from the sample but S. paratyphoid was isolated, which related to poor hygienic condition and reduced hatchability and increased embryo – death in eggs at last stage. Bruce and Drysdale (1991) reported that some bacteria are more effective in reducing hatchability than others, and proteus mirabilis , staphylococcus aureus and Group D Streptococcus spp. reduced hatchability to zero whereas other organisms such as micrococcus spp. had a less – dramatic effect on hatchability. In present study staphylococcus spp. (14.3%) , streptococcus spp. (7.1%) and Micrococcus spp.(14.3%) and proteus mirabilis (8%) were isolated and these organisms could related to decreased hatchability. In present work *Nisseria* spp. and

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Moraxella spp. were isolated but these organisms not reported by other workers (Seviour et al., Bruce j., and johnson A.L., Bruce, J. and prysdale, E.M., Nashed S.M., orajaka , L.J.E. and Mohank).

It was concluded that not easy to assess the role of microorganisms in influencing hatchability as many other factors also influence it. Some organsims are known to reduce hatchability and cause embryo mortality , while others are less understood in this respect. As organism that is present in an egg which has failed to hatch is not necessarily the cause of embryonic death as the embryo may have died from other reasons prior to the invasion and multiplication of the organsim. The defense mechanism of the eggs and embryo influence the rate of bacterial invation and multiplication.

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CONTENTS

DARKLING BEETLES (*ALPHITOBIUS DIAPERINUS*) AND THEIR LARVAE AS VECTORS FOR *SALMONELLA* AND *CAMPYLOBACTER JEJUNI* TRANSMISSION TO BROILERS

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Keywords: *Salmonella*, *Campylobacter*, *Alphitobius diaperinus*, Control

Abstract

The presence of darkling beetles and their larvae (lesser mealworms) is observed regularly in broiler houses and may play a role in the epidemiology of Salmonella and *Campylobacter* colonisation of chickens. In this study, beetles and larvae were infected in various ways with a cocktail of *Salmonella paratyphi B var. Java* and 3 *C. jejuni* strains and subsequently fed to 6-day-old individually housed chickens (3 insects per bird, 144 birds). Most chickens, fed with insects that were inoculated with Salmonella and *Campylobacter* at or until the day of feeding, became colonised within 2 and 5 days respectively. Beetles and larvae collected at a commercial broiler farm caused very limited colonisation of *Salmonella* and *Campylobacter*
In conclusion, it can be stated that beetles and larvae of *Alphitobius diaperinus* are able to transmit *Salmonella* and *C. jejuni* to chickens. It is imperative to include control of *Alphitobius diaperinus* beetles and larvae in the biosecurity system of broiler farms.

Résumé

Les coléoptères d*'Alphitobius diaperinus* (darkling beetles) et leurs larves (lesser mealworm) sont fréquemment présents dans les entreprises avicoles et ont un impact au niveau de l'épidémiologie de *Salmonella* et *Campylobacter* chez le poulet.

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Dans cette étude, des poussins de six jours placés dans des cages individuelles (3 insectes par poussin, 144 poussins) ont été nourris avec des coléoptères et des larves contaminés par *Salmonella paratyphi B var. Java* et 3 types de *Campylobacter jejuni*.
La plupart des poussins qui mangeaient les insectes contaminés par *Salmonella* et *Campylobacter* jusqu'au jour de consommation ou le jour même de consommation, étaient contaminés par *Salmonella* et *Campylobacter* au bout de 2 à 5 jours. Les coléoptères et les larves ramassés dans une ferme commerciale avicole produisaient une infection très limitée de *Salmonella* et *Campylobacter*.
En conclusion, les coléoptères et larves de *Alphitobius diaperinus* constituent des vecteurs de contamination des poulets par *Salmonella* et *Campylobacter*. Il est nécessaire d'inclure le contrôle des coléoptères et des larves dans le système de biosécurité des fermes avicoles.

Introduction

Salmonella and *Campylobacter* infections frequently occur in broiler husbandry and these pathogens are held responsible for outbreaks of human food-borne disease. Despite introduction of *Salmonella* and *Campylobacter* control programmes in The Netherlands, recent figures show that respectively approx. 10% and 40% of broiler flocks are still positive for *Salmonella* and *Campylobacter*. The control programmes contain a number of different approaches, such as biosecurity at all levels in the broiler production column, application of Competitive Exclusion in one-day-old chicks, acidification of feed and drinking water and control of rodents and insects at farm level (Bolder, 2003).
Insects that frequently occur in poultry houses are of the species *Alphitobius diaperinus* (A.d.), also called darkling beetles and its larvae lesser mealworms (Jacobs-Reitsma, et al.,1995) These insects appear to be persistent in poultry houses, and can therefore be a transmitter of different types of poultry pathogenic micro-organisms or zoonotic bacteria such as *Salmonella* and *Campylobacter*. Beetles and larvae survive in empty poultry houses by hiding in insulation material of ceilings, under floors, in hollow walls and in cracks and joints (Bolder, 2002).
It is not clear whether A.d. can be a vector in the transmission of *Salmonella* and *Campylobacter* in broilers. Contradictory results have been described regarding the survival of the pathogens in the insects (Hazeleger et al., 2001; Bang et al., 2001).
Previous experiments have shown that young broiler chicks of approx. one week old actually did consume beetles and larvae of A.d. (Jacobs-Reitsma, personnel communication).
In this paper survival of *Salmonella* and *Campylobacter* in A.d. and the transmission of both micro-organisms through A.d. to broilers is described.

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Material and Methods

Individually housed one week old broilers were fed (day 0) with either A.d. beetles or larvae that were infected with a mixed culture in Brain Heart Infusion Broth (24h, 37°C) of *Salmonella paratyphi B var. Java* (nalidixic acid resistant) and three different *Campylobacter jejuni* strains.

Five groups of A.d. beetles and larvae were prepared:

1. inoculated with a freshly prepared culture of *Salmonella* and *Campylobacter* at the day of feeding to the chicks orally (at day 0).
2. daily inoculated with a freshly prepared culture of *Salmonella* and *Campylobacter* for 4 weeks but not one week prior to feeding to the chicks orally (at day 0).
3. daily inoculated with a freshly prepared culture of *Salmonella* and *Campylobacter* for 5 weeks, feeding to the chicks orally (at day 0).
4. daily inoculated with a freshly prepared culture of *Salmonella* and *Campylobacter* for 5 weeks until the day they are fed to the chicks orally for 7 successive days (day 0-6).
5. A.d. beetles and larvae collected at a commercial broiler farm, fed to the chicks orally (at day 0).

Inoculation of A.d. beetles and larvae took place by giving drops of freshly prepared overnight cultures of *Salmonella Java* and *Campylobacter jejuni* (mix of 3 strains).

Every group was divided in two sub groups, one fed with beetles and the other with larvae. The number of chicks in groups 1a and 1b was 12, all other groups were 15 chicks. Each chick was fed 3 A.d. beetles or larvae, after feed deprivation for 2 h.

Broilers had ad lib. access to feed and water and were housed in battery cages, separated from each other by one empty cage. At the day of arrival the *Salmonella* and *Campylobacter* status of the broilers was determined.

Fresh faecal samples were collected at day 1, 2, 5, 7, 9, and 12 post infection (p.i.) from every individual chick. At the age of 20 days (day 14 p.i.) the broilers were euthanized and caeca were collected.

Samples for *Salmonella* analysis were pre-enriched in Buffered Peptone Water (24h, 37°C), followed by plating on Brilliant Green agar with 100 mg/l nalidixic acid (24h, 37°C). Samples taken from the one day old chicks at arrival, from the commercial farm and faeces samples from group 5 were also plated on Modified Semisolid Rappapport Vassiliadis agar (24h, 41.5°C) (to be able to find also nalidixic acid susceptible strains), and suspect isolates were cultured on Brilliant Green Agar (24h, 37°C), followed by biochemical and serological tests.

Faeces samples for *Campylobacter* were directly plated on Cefoperazone Charcoal Desoxycholate agar (CCDA) (48h, 42°C, micro-aerobic). Beetles and larvae were

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enriched in Preston Broth (24h and 48h, 42°C, micro-aerobic), followed by plating on CCDA.

Results and discussion

Day old broilers, feed and water were free of both *Salmonella* and *Campylobacter* at the start of the experiment.
Faecal samples from the commercial farm where A.d. beetles and larvae were collected, were *Salmonella Java*-positive and *Campylobacter*-negative. Collected beetles and larvae were *Salmonella*-positive and only larvae were *Campylobacter* positive after 48h enrichment in Preston broth.
Every recently infected batch of beetles and larvae appeared to be both *Salmonella* and *Campylobacter* positive before feeding to the chicks. *Campylobacter* could neither be isolated from the beetles and larvae from group 2, nor from the beetles from the commercial farm.
The percentage of *Salmonella*-positive chicks is presented in Table 1. The percentage of *Campylobacter*-positive chicks is given in table 2.
In group 1, both beetles and larvae are very infectious, every individual chick produced a *Salmonella*-positive faeces sample at least on one day of sampling. At three weeks of age (= 2 weeks p.i.) in caecal contents still 20% of the chicks were *Salmonella*-positive. In group 1, from day one onwards, the *Campylobacter* infection increased. Once positive, all chicks stayed *Campylobacter*-positive until the end of the experiment, which is common for *Campylobacter* colonisation in broilers. In group 2, *Campylobacter* was found only at day 12 and day 14 respectively in the beetles and larvae group, while *Salmonella* infection was limited to respectively 3 and 4 chicks.
Beetles of group 3 were very infectious for *Salmonella* and *Campylobacter*, larvae however only caused limited shedding of *Salmonella* while infection of chicks started with some delay. The difference in infectiousness between beetles and larvae cannot easily be explained, since detection of *Salmonella* and *Campylobacter* status in both was qualitatively only, and may be due to differences in numbers of *Salmonella* and *Campylobacter*, lower in larvae than in beetles. Group 4 shows that repetition of feeding infected insects leads to high and persisting infectiousness for both *Salmonella* and *Campylobacter*.
Results of group 5 suggest that either a small number of insects was *Salmonella* positive or the number of *Salmonella* cfu per insect was too low to cause subsequent colonisation of chicks, regarding the limited number of *Salmonella* positive chicks; 3 chicks in the group fed with beetles and one in the larvae group. *Campylobacter* could only be isolated from one chick, fed with beetles from day 5 onwards. Only one chick that was fed with larvae was *Campylobacter*-positive in caecal contents at day 14 p.i..

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Isolated *Campylobacter* strains will be typed by AFLP to confirm the transmission by beetles and larvae during these experiments.
In conclusion, *Alphitobius diaperinus* can play a role as a vector for *Salmonella* and *Campylobacter* transmission, both within broiler flocks, but especially between successive flocks on a farm. Therefore control of these insects as a tool to maintain biosecurity is mandatory.

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Table 1 Percentage of *Salmonella*-positive live broilers

	faeces	faeces	faeces	faeces	faeces	faeces	caeca
day p.i.	*1*	*2*	*5*	*7*	*9*	*12*	*14*
Group							
1 beetles	67	58	58	42	36	20	20
1 larvae	75	75	50	25	20	10	20
2 beetles	0	7	20	7	7	14	7
2 larvae	13	13	20	8	0	8	0
3 beetles	40	80	87	71	50	36	36
3 larvae	21	36	29	8	10	11	0
4 beetles	27	53	57	77	75	64	50
4 larvae	53	40	60	79	50	38	15
5 beetles	0	0	13	8	17	8	0
5 larvae	0	0	0	0	0	6	0

Table 2. Percentage of *Campylobacter*-positive live broilers

	faeces	faeces	faeces	faeces	faeces	faeces	caeca
day p.i.	*1*	*2*	*5*	*7*	*9*	*12*	*14*
Group							
1 beetles	58	50	75	58	82	80	90
1 larvae	33	58	75	58	80	90	90
2 beetles	0	0	0	0	0	7	7
2 larvae	0	0	0	0	0	0	17
3 beetles	67	80	87	79	86	79	86
3 larvae	14	14	36	58	40	56	56
4 beetles	27	20	71	71	92	73	100
4 larvae	33	40	73	86	100	100	100
5 beetles	0	0	7	8	8	8	8
5 larvae	0	0	0	0	0	0	6

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Session 3
Evaluation and control of risks in poultry production

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TOWARDS A GLOBAL AND BAYESIAN APPROACH TO PERFORM QUANTITATIVE RISK ASSESSMENTS IN FOOD. THE CASE OF *CAMPYLOBACTER JEJUNI* IN CHICKEN PRODUCTS

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Keywords: Bayesian, risk assessment, *Campylobacter*, chicken

Abstract

Chicken consumption can induce cases of campylobacteriosis by the ingestion of pathogen bacteria, via the meat or via cross-contamination. This is the consequence of complicated routes going through the farms, the slaughterhouses, the stores, the consumer kitchens, the reactions of individuals to the ingestion of the pathogens. We are not specialised in any of the subjects involved in these many steps, but, as statisticians and numerical model makers we propose to set a global and Bayesian way about risk assessment. We expect it to be as relevant as those described in the literature. Here we set out our arguments, hoping to show some numerical results at the conference time.

Résumé

La consommation de poulets peut conduire à des campylobactérioses, directement ou par contamination croisée sur d'autres aliments. Ces pathologies sont le résultat d'une longue chaîne d'événements se déroulant dans les élevages, les abattoirs, les commerces, les cuisines et au sein des individus ingérant les bactéries pathogènes. Bien que non spécialistes des disciplines en cause, nous proposons en tant que statisticiens et modélisateurs une approche de l'appréciation quantitative des risques qui, nous espérons, se révélera au moins aussi efficace que celles décrites dans la littérature. Il s'agit d'appréhender le système globalement de manière bayésienne. Nous détaillons ici les arguments sur lesquels se fondent nos espoirs, et tenterons de disposer de premiers éléments numériques lors de la conférence.

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This kind of disease is worth studying because of the Guillain-Barré syndrome, a rare consequence of campylobactoriosis, consisting of a demyelating disorder of the peripheral nervous system.
Figure 1 displays the main points to take into account when attempting to perform a quantitative risk assessment of campylobacterioses due to chicken meat consumption. Such description can be found with many details into the two technical reports recently published by the Danish Veterinary and Food Administration and FAO/WHO (see references). As for many of the food borne diseases, the chain of events described here comprises the production and consumption blocks whose outputs are the inputs of the exposure block, itself followed by a pathology block quantifying the occurrence of the disease. This diagram is very schematic. For the specialists, every step has to be split into more steps, each variate needs to be described by several scalar variates and more complicated relationships have to be introduced. Nevertheless, if we produce a much more complicated diagram, such a graph construction will still be inadequate for several essential reasons.

- The main reason is that it is not indicated if we are interested in the destiny of one random chicken or of one random consumer (or one meal comprising chicken meat), or in the campylobacteriosis cases of a given population of human individuals during a given period. The difference can be difficult to grasp because the expectation probability for one individual to become ill is equal to the expectation of the number of ill people divided by the number of people. It lies in the correlations existing between the status of chickens (for instance as a consequence of the farms or the slaughterhouses) or individuals (for instance belonging to the same family, or going at the same shop) changing the variances.
- The spatial and temporal nature of the phenomenon is not expressed. Main trends and aggregative behaviours are ignored distorting the model.
- Most of the steps are not singular but plural. For instance, slaughterhouses are numerous, distinct and different.
- A very strong feature is the lack of reliable and homogeneous data at each step. The consequence is disastrous for most standard statistical data analysis.

Finally, it is worth noticing that Campylobacter is not able to proliferate outside the alimentary canal, so there is no need to model the increase in the bacteria population after the slaughterhouse step; this is an important and simplifying feature.

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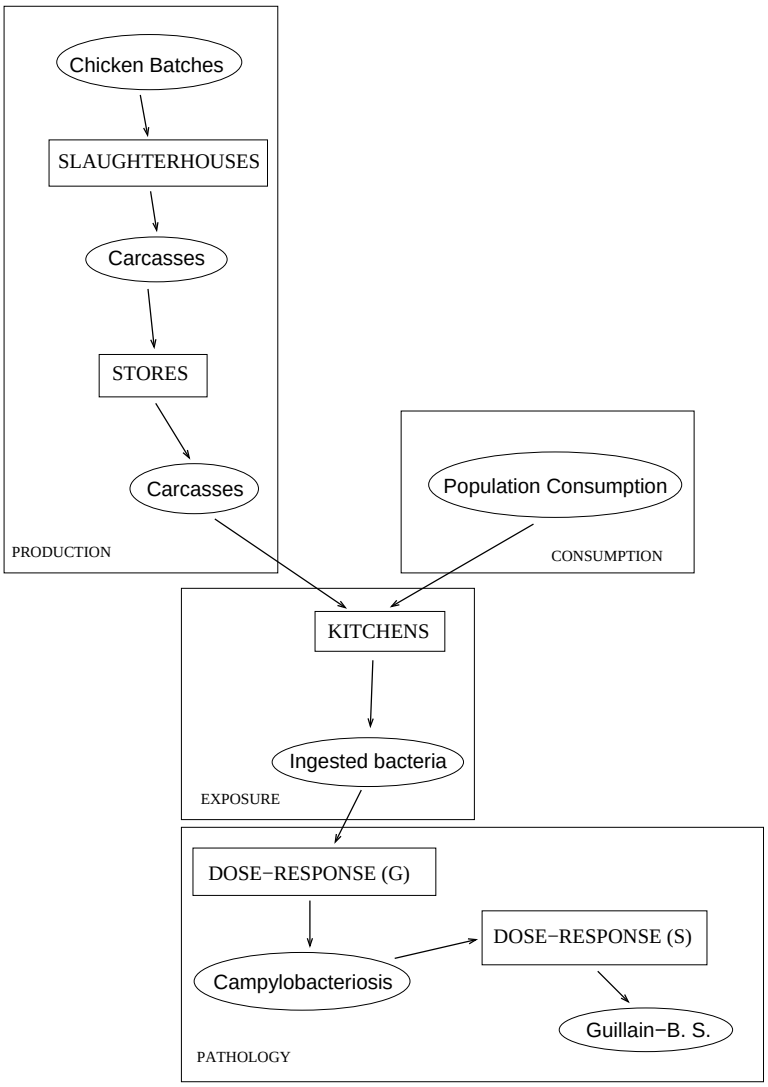

Figure 1: Main steps [rectangles] and variates (ellipses) and their relationships {arrows} involved in the generation of campylobacteriosis cases.

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Material and Methods

In order to deal with the chain described in Figure 1, it seems to us that one has to admit that some parts of the model are probabilistic. Random variates are an efficient way to model inherent variability or to express inevitable uncertainty. For instance, they can be used to solve the *plural* problem of the steps previously mentioned. We can propose that a slaughterhouse is sampled from the population of possible slaughterhouses and, as a consequence, its characteristics follow some distribution probability depending of one or several parameters.

However, one must immediately add that, due to (i) the complex relationships between variates and (ii) the heterogeneity and the weakness of the available information, standard statistical analysis is not straightforward. Indeed, an inference approach takes its value from well formed data sets, and is difficult to deal with intricacies. For example, it is not an easy task to incorporate into the model that a given parameter lies between two more or less known values.

On the contrary, the Bayesian approach performs this kind of task quite naturally. The reason is that the paradigm is different. The Bayesian analysis can be seen as a successive aggregative process of new pieces of information leading to a complete and standalone description of the phenomenon. The counterpart is that the first complete description must be established at the start even in a non-informative manner. In Bayesian terminology, it is called the *prior*, its transformation with the help of data is called the *posterior*; the posterior is the natural prior for a next step. From a technical viewpoint, the prior can be compared to the specification of a Monte-Carlo simulation study, except that for some parameters, values are only given through distributions depending on "hyperparameters".

Another purpose is to conduct a global analysis. There are no precise statistical reasons to do so, only some common sense. The opposite way is to independently model each identified module before proceeding to their assembling. The dangers of this strategy are to go unnecessarily deeper in some modules, and to neglect key modules for the final result. A global approach focuses on the balance between modules, and emphasizes the final aim. Another important argument is that through the relationships existing between modules, we can hope for a better fit of a given module by means of information available in other modules. The typical case is the dose-response module for which data is quite small, and not very relevant because the volunteers used in experiments are generally healthy individuals and not representative to the target population.

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Results and discussion

The construct we propose involves many difficulties.

- Even if the Bayesian framework is well adapated to integrate heterogeneous data sets, we will be confronted with two kinds of difficulties. (i) Most information is not available as data sets but as "feelings of experts". By some discussions this can be translated into distribution probabilities but not in data sets. (ii) How to separate which part must be used to define the prior modelling from the information to consider as data? The easy answer is to assign expert feelings to the prior but this asks for further reflection.
- Contradiction within material will be appeared and we will need the advices of experts to decide which to discard, and which relative weights to give.
- It is not simple in Bayesian framework to select a well adapted model. The model must be as simple as possible to produce robust predictions but at the same time it must be sufficiently realistic to answer the questions relevantly. We have in mind that the aim is to give the experts a tool for making decisions, and that precise behaviour of each variate is not the goal; indeed, the expert will add her/his personal interpretation and implicit knowledge.
- We are not specialists of any fields involved in the chain but there is no one who is an expert in all these fields. This implies constant and close exchanges between many participants. Each one must explain and understand the others. We hope that a numerical software application could be a way to facilitate the achievement of this target.

Finally, we would state that the construction of a quantitative model is the best way to interact efficiently between specialists of different fields, perhaps before proposing numerical experiments.

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CONTENTS

BACTERIAL EGGSHELL CONTAMINATION IN THE EGG PRODUCTION CHAIN AND IN DIFFERENT HOUSING SYSTEMS

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Keywords: bacterial contamination, eggshell, quality assurance, consumption eggs

Abstract

Washing eggs in sterile plastic bags with diluent is an efficient sample preparation method for the determination of the bacterial contamination on eggshells. The total count of aerobic bacteria and the total count of Gramnegative bacteria on the eggshell can be used to detect critical contamination points in the egg production chain. The number of eggs to be sampled in a point of the production chain was determined on a statistical basis and fixed on 40 for non-graded eggs and on 20 for graded eggs. In two production chains, one cage production and one organic production system, critical contamination points were identified. The influence of the housing system on the bacterial contamination of the eggshell at the stable was studied. A positive correlation was found between the initial bacterial eggshell contamination and the concentration of bacteria in the air of the poultry houses. With the exception of heavily soiled shells, like shells from ground eggs, there is a poor correlation between the level of bacterial contamination and the visual eggshell contamination.

Résumé

Le rinçage des œufs dans un milieu de dilution est une méthode efficace pour évaluer la qualité micro biologique de la coquille. Le dénombrement de la flore aérobie mésophile et des bactéries à coloration de Gramnéptive est une méthode qui peut être utilisée pour déterminer les points critiques tout au long de la chaîne de production d'œuf. Le nombre d'œufs à analyser à chaque point, a été déterminé par une méthode statistique et fixé à 40 pour des œufs non triés et 20 pour des œufs triés. Les points

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critiques ont été identifiés pour un système de production en cage et un élevage biologique. L'influence des conditions d'élevage sur la qualité micro biologique de la coquille a été déterminée. Une corrélation positive entre la contamination de la coquille et la contamination bactériologique de l'air a été établie. A l'exception des œufs souillés (perdus au sol) la corrélation est faible entre la contamination bactérienne et l'aspect visuel de l'œuf.

Introduction

External eggshell contamination could be important for the shelf life and the food safety of consumption eggs and egg products. In literature few data are published about the bacterial contamination on the shell of consumption eggs. The shell can already be infected when passing through the vent, but many researchers suggest that the main contamination occurs within a short period after laying due to contact with dirty surfaces (Harry, 1963; Board *et al*., 1964; Quarles *et al*., 1970 and Gentry and Quarles, 1972). It is hypothesized that bacterial contamination of the internal egg content could be the result of the penetration of the shell by bacteria deposited on the surface of the egg after it has been laid (Haines, 1938; Harry, 1963; Schoeni, *et al*. 1995). Because of the introduction of different alternative housing systems for laying hens, the study of the bacterial contamination on the shell of consumption eggs is important.
The aim of our study was to develop the methodology to quantify the bacterial contamination on the eggshell and to detect critical points of contamination in the entire production chain; this means from the production, grading and transport up to the sales-outlets. Also the influence of the housing system on the bacterial contamination of the eggshell of consumption eggs was studied. A full paper with the results was submitted to Food Control.

Material and Methods

Determination of bacterial eggshell contamination
Different methods for the recuperation of bacteria from the eggshell, different counting media and incubation temperature/time combinations were compared. Based on these results the most efficient and practical method was used for application in further experiments.

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Number of samples and statistical analysis of data

To produce statistically reliable results, a minimum of eggs need to be sampled at a certain point in the production chain. The minimum number of samples is based on the number of samples from which the standard error starts converging to an asymptotic value (Grijspeerdt and Verstraete 1997). Standard differences were assessed using an analysis of variance (ANOVA).

Collection and transport of eggs

In the points of the production chain before packaging, the eggs were picked up with the fingertips and placed in new carton filler-flats. Between each sampling point the fingertips were disinfected. In the points after packaging closed cartons (first category eggs) or carton filler-flats (second category eggs) filled with eggs were sampled. The eggs were brought by car, in ambient conditions, to the laboratory were they were kept for maximum 56 hours in ambient conditions before analysing.

Influence of time, temperature and atmospheric humidity on the bacterial eggshell contamination

The influence of storage of the eggs till expire date at room temperature with an average atmospheric humidity of 50% and in a refrigerator at 5°C with an average atmospheric humidity of 85% was studied.

Sampling through the production chain

Sampling was performed in a caged layer house and an organic production unit, housing respectively the brown-shell breeds Warren Sex A Line and Bovans Goldline hens. At least 7 points were sampled in the entire production chains.

Influence of the housing system on the eggshell contamination

To study the influence of the housing system on the bacterial eggshell contamination, three different systems; enriched cages, perchery and cage production, arranged on the same farm were followed during several months.

Results and discussion

Determination of bacterial eggshell contamination

The most efficient and practical method for the recuperation of bacteria from the eggshell was based on a washing procedure of the intact egg in a plastic bag with 10 ml diluent. The diluent was plated by a spiral-plater on Nutrient Agar for the determination of the total count of aerobic bacteria and on Nutrient Agar with 0,0001% crystal violet for the total count of Gramnegative bacteria. Plates were incubated for 3 days at 30°C.

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Number of samples

To produce statistically reliable results, minimum 40 eggs need to be sampled at a certain point in the production chain with non-graded eggs. The required number of samples for graded eggs was set at 20.

Collection and transport of eggs

Collecting the eggs by hand did not influence the results significantly. Also no significant difference in the bacterial flora was found on eggs examined immediately and after keeping for 56h in ambient conditions at the laboratory.

Influence of time, temperature and atmospheric humidity on the bacterial eggshell contamination

Contrary to the total count of aerobic bacteria, the Gramnegative bacteria on the eggshell decreased statistically significantly at room environment (from 4.04 to 3.23 log cfu/eggshell) but not at refrigeration environment (from 4.04 to 3.66 log cfu/eggshell). This was probably due to the lower humidity at room temperature.

Sampling through the production chain

Cage production:

Figure 1 shows an increase in total count of aerobic and Gramnegative bacteria, at the moment the eggs enter the grading, candling and packing area ('metal mat' in Figure 1). For both parameters the increase was statistically significant (ANOVA). This point in the production chain was indicated as a critical control point.

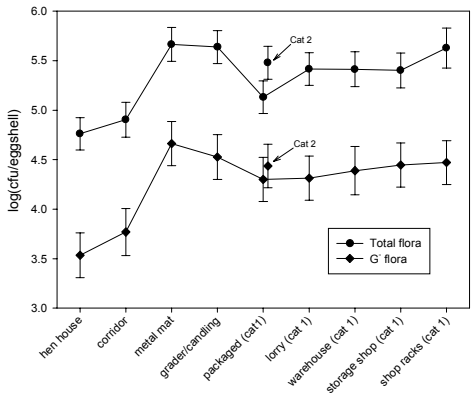

Figure 1: The total count of aerobic (Total flora) and Gramnegative (G^-flora) flora in the different points of the cage production chain.

Organic production:

Compared to the caged layer house, the bacterial eggshell contamination through the organic chain showed less fluctuations. The initial contamination with aerobic bacteria

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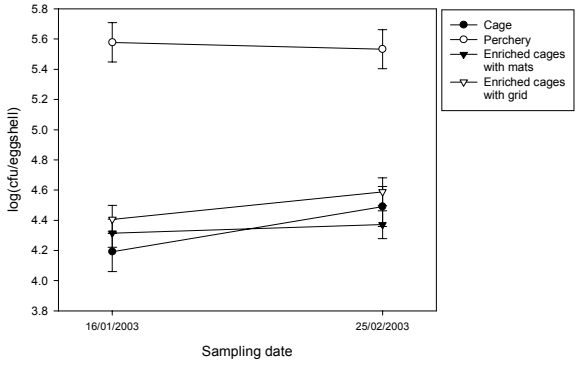
(5.8 log cfu/eggshell) of the eggs on the covered conveyor of the nest boxes was 1 log higher compared to the eggs on the conveyor of the stable in the cage production. This raise of initial contamination makes the nest boxes in the organic housing system a critical point for bacterial eggshell contamination. The higher initial contamination was also reflected in the air where a much higher contamination (2.2×10^4 cfu/50l air) was measured compared to the caged stable (1.4×10^3 cfu/50l air).
Despite the clear difference in critical control points for bacterial contamination, the total bacterial count on the eggshell for the 2 production systems was comparable at the end of the chain. With the exception of heavily soiled shells, like shells from ground eggs, a poor correlation was found between the level of bacterial contamination and the visual eggshell contamination.
Influence of the housing system on the eggshell contamination
During august 2001 till june 2002 the bacterial eggshell contamination, total count of aerobic bacteria and Gramnegative bacteria, was analyzed bimonthly for eggs from a cage production and enriched cages with nest boxes containing mats or grids, all productions arranged on the same farm with the same hen breed. In a period of 8 months no statistical differences were found between the three housing systems. Only on the last sampling date the eggshell contamination at the cage production was significantly higher. More pronounced was the seasonal influence on the eggshell contamination with a statistically significant decrease in the winter period.
A second study is running, including also a perchery system. The first results indicate a statistically higher contamination of the eggshells, with total aerobic flora, of the perchery system compared to the cage production and the enriched cages. The first data are shown in figure 2.

Figure 2: Influence of the housing system on the bacterial eggshell contamination (Total flora).



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In all studied poultry houses a positive correlation was found between the initial bacterial eggshell contamination and the concentration of bacteria in the air of the poultry houses.

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RISK FACTORS ASSOCIATED WITH *SALMONELLA ENTERICA* SUBSP. *ENTERICA* AND *CAMPYLOBACTER* SPP. CONTAMINATION OF BROILERS AND CHICKEN CARCASSES IN SENEGAL

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Keywords: *Salmonella*, *Campylobacter*, Poultry, Risk factors, Senegal

Abstract

The objective of this study was to identify the risk factors for *Salmonella* and *Campylobacter* contamination in Senegalese broilers and chicken carcasses. Seventy broiler farms and 120 traditional abattoirs were studied from January 2000 to December 2002 around Dakar. 28.6% of the 70 flocks tested positive for *Salmonella* and 62.9% for *Campylobacter*. 43.3% of the 120 carcasses batches tested positive for *Salmonella* and 69.2% for *Campylobacter.* The most prevalent serovars isolated were *S.* Hadar and *S.* Brancaster. Only two species of *Campylobacter* were isolated: *C. jejuni* and *C. coli*. Most of the risk factors identified deal with hygienic practices and biosecurity measures: thorough cleaning, disinfection and hygienic routines have to be implemented.

Résumé

L'objectif de cette étude était d'identifier les facteurs de risque de contamination par *Salmonella* et *Campylobacter* des poulets de chair et de leurs carcasses. 70 exploitations avicoles et 120 abattoirs traditionnels, autour de Dakar, ont ainsi été étudiés entre Janvier 2000 et décembre 2002. 28.6% des bandes de poulets étaient contaminées par *Salmonella* et 62.9% par *Campylobacter*. 43.3% des lots de carcasses étaient contaminés par *Salmonella* et 69.2% par *Campylobacter*. S. Hadar et S. Brancaster ont été les deux sérovars prédominants. Seules deux espèces de *Campylobacter* ont pu être isolées : C. jejuni et C. coli. La plupart des facteurs de risque identifiés tiennent aux pratiques hygiéniques et aux mesures de biosécurité: des

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procédures de décontamination minutieuses et des attitudes hygiéniques doivent être mise en œuvre.

Introduction

Infections with *Salmonella* or *Campylobacter* are ones of the most common causes of gastroenteritis worldwide. In developing countries, these mircroorganisms are a source of common complications in HIV-infected patients. Most infections are acquired by the consumption of poultry meat products. The effective prevention of these infections requires a control along the food production chain, mainly at the farm and the abattoir levels.
Numerous studies have investigated the origin of contamination in poultry but very few have been done in developing countries. In Africa particularly, where modern poultry production is recent, a better understanding of the epidemiology of *Salmonella* and *Campylobacter* in chickens in Africa is essential to provide a basis for a more specific control strategy. Therefore, the aim of the present study was to assess the association of managerial practices in farms and abattoirs with the contamination of broilers and chicken carcasses in Senegal.

Material and Methods

1 Study sample:
Our study was carried out from January 2000 to December 2002 and involved 70 broiler farms and 120 traditional slaughterhouses around Dakar. Only one flock was studied on each farm and only one batch of 5 chicken carcasses on each abattoir. The farm and slaughterhouse selection was based on the owner's willingness to cooperate. The slaughtering process in these abattoirs was manual and rudimentary, and frequently the hygienic conditions were poor.
2.2 Data Collection :
-Each farm was visited four times. The first visit occurred by the end of the previous flock before slaughtering to define the *Salmonella* status. The second visit occurred during cleaning and disinfection procedures. The third and the fourth visits took place during the lifespan of flock at the beginning and at the end of the rearing period. At the first (previous flock), third (one day-old chicks) and fourth visits (end of the rearing period), twelve pooled samples of five fresh droppings were taken to assess the *Salmonella* status of the flock. *Campylobacter* status of the flock was also assessed at the fourth visit. Data concerning farm and house characteristics, conditions of chicks

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placing, management of ill and dead birds, control of rodents and other domestic animals, feeding and watering practices, farm staff and visitors, cleaning and disinfection procedures were collected from a questionnaire filled with each farmer.
-Each slaughterhouse was visited once. During each visit, a batch of five chicken-broilers to be slaughtered was randomly chosen. Cloacal swabs were taken to assess the *Salmonella* and *Campylobacter* status of these live birds. After slaughtering, breast skin samples (weighing 25 g) were removed from the five chicken carcasses of the same batch to assess their status. Data concerning slaughtering characteristics, feed withdrawal, bleeding, scalding, defeathering, evisceration, handling, storing and preservation, slaughterhouse staff, cleaning and disinfection procedures were collected from a questionnaire filled with each slaughterer.
2.3 *Salmonella* and *Campylobacter* isolation and identification: the bacteriological methods have been described in (Rose et al., 1999) and in (Refregier_Petton et al., 2001). PCR method has been used for *Campylobacter* species identification according (Denis et al., 2001).
2.4 Definition of outcome variable
The unit observation was the flock and the batch of carcasses. A unit was declared contaminated only if one or more samples tested positive. The outcome variable was thus dichotomous (contaminated *vs* non-contaminated).
2.5 Definition of explanatory variables
All variables (31 for the farm level and 31 for the abattoir level) were encoded categorically. The number of categories per variable was limited, so that frequency rates of categories were >10%. These variables were selected from a preliminary step aimed at lowering the chance of obtaining results affected by multicollinearity in the data set (Dohoo et al., 1996). All bilateral relationships between possible explanatory variables were checked ($\chi 2$). For all bilateral relationships between variables evidencing strong structural collinearity, one of the two variables of interest (the one most related to the outcome variable) was chosen.
2.7 Statistical Procedure
A two-stage procedure was used to assess the relationship between explanatory variables and *Salmonella* and *Campylobacter* status of the flock. Logistic regression was used according to the method described by Hosmer and Lemeshow (2000). In the first stage, a univariable analysis was performed to relate *Campylobacter* and *Salmonella* contamination of the flock to each explanatory variable. Only factors associated with *Campylobacter* contamination of the flock (Pearson $\chi 2$ test, $p<0.25$) were included in a full model for multivariate analysis. The second stage involved a logistic multiple regression model which included all factors that passed the first screening test. The contribution of each factor to the model was tested using a likelihood ratio $\chi 2$ test through a step by step procedure (backward and forward). Odds ratios were converted into relative risks according to the method proposed by

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Beaudeau and Fourichon (1998). Interactions were not tested (because of the small sample sizes).

Results and discussion

28.6% of the 70 flocks studied tested positive for *Salmonella* and 62.9% for *Campylobacter*. 43.3% of the 120 carcasses batches studied tested positive for *Salmonella* and 69.2% for *Campylobacter*

The most prevalent serovars isolated were *Salmonella* Hadar and *Salmonella* Brancaster. Only two species of *Campylobacter* were isolated: *C. jejuni* and *C. coli*.

The variables tested in the screening analysis significantly associated with contamination are gathered in table 1.

Although the study included a random selection of broiler flocks and slaughterhouses, the required willingness of farmers to cooperate during the lifespan of the flock might have led to a selection bias. Nevertheless, given that there were an estimated 600 broiler flocks and 175 slaughterhouses in the country, our sample was representative of most chicken broiler flocks and abattoirs located in Senegal. The use of questionnaires for collection of baseline data could also introduce bias, but most questions were objective and closed. For subjective questions, a detailed description for each of the categories was provided. Moreover, the data collection by only two people trained for this work for the study's duration contributed to the high precision of the results.

- Risk factors at the farm level :

The *Salmonella* status of the previous flock living in the same poultry house was a significant risk factor for a new flock to be *Salmonella* contaminated. As Lahellec et al. (1986) indicated, if a broiler flock is infected by S. *enterica*, the bacteria can persist if the prerequisites and procedures for cleaning and disinfection are not adequate. Furthermore, *Salmonella* contamination of day-old chicks was found to be a risk factor for *Salmonella* contamination in agreement with (Christensen et al., 1997) *Salmonella* can be vertically transmitted but this route of transmission is mainly associated with S. Enteritidis; horizontal transmission from the hatchery and the house environment was reported as the main route (Fris and Van Den Bos, 1995).

Use of antimicrobial drugs on day-old chicks decreased the risk of *Salmonella* contamination. Prophylactic use of antibiotics against mortality during the first days of life may reduce the number of colonized and shed bacteria in the surroundings (Chriel et al., 1999). This practice may be useful to prevent the spread of infection at the farm, particularly in the absence of vertical infections routes.

Keeping ill birds in the farm increased the risk of *Salmonella* contamination. Infections of poultry with immunosuppressive microorganisms can increase the ability

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of *Salmonella* to colonize the intestinal tracts of chickens (Gast, 1997). So, sick birds should be removed because they could be a source for *Salmonella* contamination of the other birds of the flock. Keeping other animals in the farm, such as laying hens or cattle, also increased the risk of *Campylobacter* contamination. These animals are often found to be permanent carriers of *Campylobacter* (Jacobs-Reitsma, 1997).
We also found that the risk of *Campylobacter* contamination was increased when the farm staff did not wear specific clothes exclusively for working in poultry houses. Farm workers could be a possible source of the organism, not acting as a direct source but as mechanical vehicles of *Campylobacter* contamination from different sources such as other farm animals (Jacobs-Reitsma, 1997). That's why other poultry farmers could increase the risk of *Salmonella* contamination.
Using a detergent for cleaning decreased the risk for a flock to be *Salmonella* contaminated. Cleaning is an essential stage for the removal of organic and inorganic debris from a surface which might support micro-organisms and provide insulation that reduces the efficiency of disinfecting procedures. But most of poultry producers in Senegal did not perform any thorough cleaning and they had the feeling they were reaching a sterile condition because they used disinfectants, when they could only achieve a sanitized condition at the very best.
In the same way, poor maintenance of poultry house surroundings increased the risk of *Campylobacter* contamination because the main source of *Campylobacter* is the environment (Van de Giessen et al., 1998).
The risk of *Campylobacter* contamination also increased when manure disposal was not outside of the farm. In Senegal, some farmers use manure for market gardening on the farm, but manure may play a role in maintaining a cycle of contamination in the environment. *Campylobacter* tend to die out rather than multiply in an environment under normal conditions (Genigeorgis et al., 1986), but transmission of the organism to subsequent flocks may occur if the old manure is cross-contaminated.
Poultry houses without cemented floors were associated with an increased risk of *Campylobacter* contamination. Cleaning is easier on cemented floors for the removal of organic and inorganic debris from a surface which might support micro-organisms and provide insulation that reduces the efficiency of disinfecting procedures. And *Campylobacter* only survive in poultry houses when cleaning and disinfection are inadequate (Evans and Sayers, 2000).

We found that using cardboard boxes as feed plates for transporting the chicks from the hatchery was increasing the risk of *Campylobacter* contamination. No studies have mentioned such a risk factor because most of the world's poultry farmers use specifically designed feed plates for chicks which are easy to clean. In Senegal, because of the cost of these imported specific feed plates, many farmers use cardboard boxes to feed birds during the first two or three weeks of the rearing period. As Achen et al. (1998) showed, *Campylobacter* shedding starts as early as 2-3 weeks of age.

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Subsequently, feed could have been contaminated with excreta and spread contamination to the birds. This hypothesis contradicts the general acceptance that no vertical transmission of *Campylobacter* can occur in poultry (Shane, 1992), contrary to Cox et al. (2002)

Risk Factors at the abattoir level:

The *Salmonella* and *Campylobacter* status of the live broilers birds before slaughtering was a significant risk factor for the carcasses contamination after slaughtering. This result confirms the general acceptance that broilers become first colonised at the farm level, mainly through the environment (Salvat, 1997)

Feed withdrawal before slaughtering decreased the risk of *Salmonella* contamination. Withholding feed is a means of reducing the natural distension of the intestines and hence the risk of gut breakage during processing (Mead, 1980). In Senegal, evisceration is manual and the intestinal tract is likely to break in case of absence of feed withdrawal, increasing the possibility of carcass contamination with *Salmonella* derived from guts contents. In modern abattoir, evisceration is automated; which offers the prospect of a better hygiene control because it eliminates the human error in manual evisceration and provides the possibility of washing and disinfecting the equipment after eviscerating each carcass (Salvat, 1997).

Scalding by water immersion is associated with an increasing risk of *Salmonella* and *Campylobacter* contamination for the poultry carcasses. Scalding has long been identified as a cross-contamination stage (Lahellec and Meurier, 1973) In fact, the birds carry large numbers of microorganisms on the skin and feathers and in the faeces, and many of these organisms enter the water as birds are moved continuously through the scald tank (Mead, 1980). In Senegal, scalding water is heated till 50°C – 60°C and then used for processing. The extent to which organisms accumulate in the scald water is explained by the continuous decreasing of the temperature of scalding and the addition of fresh water during the process.

The risk of *Salmonella* and *Campylobacter* contamination was also increased when there was not a specific worker for each slaughtering stage, particularly when the slaughtering stages were not divided. Many studies showed that plant workers could cross-contaminated chicken carcasses (White et al., 1997). When the same person has to deal with all the processing operations, he is in close contact with broiler contaminated area: skin and feathers during plucking, guts contents during evisceration; thus, his hands could contaminate the carcass by the end of slaughtering. The operative could also transfer pathogens from a contaminated bird to one that was previously uncontaminated, increasing the proportion of carcasses carrying the organism (Mead, 1980).

Thorough cleaning and disinfection procedures were significantly related to a decreasing risk of *Salmonella* contamination of chicken carcasses. Regular schedules of cleaning and disinfection of the slaughter processing plant and equipment are essential for maintaining sanitary conditions (White et al., 1997). In Senegal, these

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procedures are difficult to respect when slaughtering takes place outside, directly on the ground. Moreover, the traditional slaughterhouses do not always have drinking water for cleaning. And without the ability to clean a piece of equipment well, the process of disinfection is of little value in view of the high levels of soiling which can occur. After cleaning, disinfection is the second step which reduces the number of viable micro-organisms such as *Salmonella*.

Contact between viscera and carcass after evisceration increased the risk for *Campylobacter* contamination of the chicken carcasses. *Campylobacter* are carried in the intestines of the contaminated birds (Altekruse, 1998); so, any contact between the contaminated intestinal contents and the carcasses could be of source of cross-contamination.

Storing the chicken carcasses with protective package by the end of slaughtering reduced the risk of *Campylobacter* contamination. Packaging of poultry meat and products protects them from undesirable factors (Hafez, 1999) such as *Campylobacter* cross-contamination from the environment: equipment, hands of operatives, processing facilities, other live broilers.

Most of the risk factors deal with hygienic practices and biosecurity measures: thorough cleaning, disinfection and hygienic routines have to be implemented. To enhance the hygienic level of poultry production in Senegal, the farmers and the slaughterers must be informed and sensitized.

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Table1: Final logistic regression model for risk factors for *Salmonella* contamination of broiler flocks (70 flocks) and chicken carcasses (120 slaughterhouses) in Senegal

Variables		Logistic regression model[a]			
	% of *Salmonella* + flocks [b]	OR [c]	90% CI [d] (OR)	RR [e]	90 % CI (RR)
Salmonella status of previous flock					
S + [f]	62.5	6.82	1.16 – 40.18	3.25	1.11 – 7.05
S -	18.5	1		1	
monella status of day-old chicks					
	61.5	3.73	0.62 - 22.48	2.79	0.67 – 11.09
	21.1	1		1	
of antibiotics at Day 1					
Yes	24.6	1		1	
No	55.5	5.98	0.81 – 44.21	4.40	0.86 – 30.11
Ill birds management					
Kept in the farm	46.4	5.32	1.11 – 25.63	2.37	1.06 – 4.14
Eliminated	16.7	1		1	
Frequent poultry farmers visits					
Yes	48.3	5.38	1.05 – 27.85	3.27	1.04 – 11.38
No	14.6	1		1	
Use of detergent for cleaning					
Yes	11.6	1		1	
No	55.5	6.16	1.28 – 29.58	4.08	1.19 – 16.77
Salmonella status of broilers before slaughtering					
S + [d]	68	3.01	0.96 – 9.41	1.78	0.98 – 2.49
S -	36.8	1		1	

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Feed Withdrawal					
Yes	23.8	1		1	
No	47.5	3.75	0.65 – 21.4	1.84	0.77 – 2.76
Specific people for each slaughtering stage					
Yes	10.5	1		1	
No	49.5	10.49	1.88 – 58.52	2.40	1.38 – 2.90
Use of scalding during slaughtering procedures					
	58.8	4.51	1.6 – 12.7	2.27	2.86
	31.9	1		1	
ning after slaughtering					
	11.5	1		1	
	76.3	8.52	2.38 – 30.48	3.5	1.64 – 8.05
Disinfection after slaughtering					
Yes	4.5	1		1	
No	52	7.83	1.17 – 71.9	2.31	1.09 – 3.10

b : *Salmonella* contaminated flocks at the end of the rearing period
c: Odds Ratio
d: Confidence Interval
e : Relative risk obtained according to Beaudeau and Fourichon (1998)
f : *Salmonella* status (S+: *Salmonella* contaminated; S-: *Salmonella* free)

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Table 2 : Final logistic regression model for risk factors for *Campylobacter* contamination of broiler flocks (70 flocks) and chicken carcasses (120 slaughterhouses) in Senegal

Variables		Logistic regression model[a]			
	% of *Campylobacter* + flocks [b]	OR [c]	90% CI [d] (OR)	RR[c]	90 % CI (RR)
Poultry house with cement floor					
Yes	42.8	1		1	
No	82.9	4.15	0.91 – 18.91	1.67	0.97 – 2.62
ɛr animals in the farm					
	70.7	7.52	1.25 – 45.36	2.22	1.09 – 4.38
	51.7	1		1	
Specific clothes for farm workers					
Yes					
No	50	1		1	
	78.1	4.52	1.08 – 18.82	1.76	1.03 – 2.87
Use of cardboard box as feed plate					
Yes					
No	75.5	5.28	1.11 – 25.10	2.00	1.04 – 4.26
	40	1		1	
Manure disposal:					
Inside the farm	83.3	15.57	189 – 128.27	1.70	1.23 – 1.80
Outside the farm	58.6	1		1	
Cleaning and disinfection of house surroundings					
Yes	38.5	1		1	
No	77.3	6.86	1.06 – 44.36	1.78	1.02 – 2.28

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Campylobacter status of the birds before slaughtering					
C + [d]	89.3	8.55	2.68 – 27.59	1.80	1.34 – 2.18
C -	49.2	1		1	
Division of the slaughtering stages					
Yes	35.3	1		1	
No	74.7	4.49	0.97 – 15.35	1.36	1.02 – 1.51
Scalding during slaughtering					
	84.3	1		1	
	57.9	6.86	2.28 – 23.76	1.64	1.27 – 1.94
tact between viscera and asses					
	90.6	5.27		1	
No	57.1	1	1.49 – 18.65	1.51	1.13 – 1.76
Storing carcasses at the slaughterhouse					
No protective package	76.4	3.62	1.09 – 12.01	1.61	1.03 – 1.86
Protective package	48.4	1		1	

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COMPETITIVE EXCLUSION TREATMENT TO PREVENT *CAMPYLOBACTER* COLONIZATION OF CHICKS ISABROWN INFLUENCE OF THE ORIGIN OF THE C.E. FLORA

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Keywords : Competitive Exclusion, Colonization, *Campylobacter,* Layer

Abstract

Several Competitive Exclusion floras have been previously administered to broilers chicks to prevent *Campylobacter* contamination of chicken. Collected from adult birds, caecal or mucosal floras very often were ineffective in reducing broiler chick colonization. We decided to evaluate caecal or mucosal floras of different origin in their ability to prevent *Campylobacte*r colonization of a layer strain "Isabrown": caecal floras collected from two different flocks of young hens and a commercial product "Mucosal Starter Culture"(MSC). In three trials, after inoculation (130 to 360cfu), *Campylobacter* could never be detected from male Isabrown treated with caecal floras issued from one flock of young laying hens Isabrown but they were from chicks treated (in one trial) with MSC. Unexpectedly, in a fourth trial, *Campylobacter* did not colonize the positive controls but they did for the birds treated with a caecal flora collected from another flock of young laying hens.

Résumé

Différentes flores d' Exclusion Compétitive ont été testées pour limiter la contamination des poulets de chair par *Campylobacter*. Collectées à partir d'animaux adultes, ces flores caecales ou mucosales se sont souvent avérées inefficaces pour prévenir la colonisation de poulets de chair. Nous avons donc évalué des flores caecales ou mucosales de différentes origines pour leur capacité à limiter la colonisation par *Campylobacter* de jeunes coquelets Isabrown mâles de pondeuses : des flores caecales collectées à partir de deux lots de jeunes pondeuses et une flore commerciale "Mucosal Starter Culture"(MSC). Au cours de trois essais, après inoculation (130 à 360cfu) aucun *Campylobacter* n'a été détecté à partir des poussins Isabrown auxquels nous avions préalablement administré des flores caecales provenant

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d'un lot de jeunes pondeuses. Par contre au cours d'un de ces essais, ces bactéries ont été détectées sur les animaux traités avec la flore MSC. Dans le 4ième essai contre toute attente, les *Campylobacter* ne se sont pas implantés chez les témoins positifs alors qu'ils l'ont fait pour les animaux traités avec une flore caecale provenant d'un autre lot de jeunes pondeuses.

Introduction

Because of the increasing human campylobacteriosis, *Campylobacter* is considered as the main bacterial pathogen responsible for human gastrointestinal infections. In several studies, poultry meat , by handling or consumption of undercooked products is strongly associated with foodborne diseases due to *Campylobacter*. Consequently it is necessary to control contamination at the different steps of the poultry production. Delivering chicken flocks free of *Campylobacter* to slaughter where cross contamination are observed can contribute to decrease the number of contaminated products. The application of hygienic measures all over the rearing period seems to be one important way to prevent chicken contamination. Unfortunately, from time to time, sanitary barriers are not respected and *Campylobacter* from the environment can enter the rearing houses and consequently colonize the chicken. Others measures as the use of Competitive Exclusion floras have been considered to prevent chicken colonization by *Campylobacter*. Caecal or mucosal floras collected from healthy adult chicken were administered to young chicks before intestinal flora settlement (Shanker *et al.* 1988, Stern 1994, Mead *et al.* 1996). In previous studies (unpublished data), we tried to prevent *Campylobacter* colonization of broilers using a mucosal C.E. flora collected anaerobically from the the caeca of adult S.P.F. chicken. This flora was ineffective to prevent contamination of broiler chicks reared in isolation units. A *Campybobacter i*s scarcely detected from birds during the two first weeks of the rearing period we decided to evaluate caecal floras collected from young birds (i.e.3, 7, 10 and 14 days old) instead of adult birds ; young birds could be less sensitive to *Campylobacter* colonization because of the immature flora of the first fifteen days. When these "young" caecal floras were administered to broilers only a slight delay of colonization of the chicks was observed but they seemed to be efficient when chicks of a layer type are concerned (Laisney *et al.*, 2003). So we decided to evaluate a commercial product " Mucosal Starter Culture " and caecal floras collected from two different flocks of young hens for their ability to prevent *Campylobacter* colonization of chicks of a layer strain (Isabrown).

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Material and methods

The first part of the experiment consisted in collecting from young donors caecal material used further as a competitive exclusion treatment. From two flocks of laying hens Isabrown, several batches of C.E. floras were collected anaerobically the 3rd, 7th,10th, 14th and 24th day of the rearing period. Briefly : after sacrificing the birds, the caeca were aseptically removed placed and immediately introduced in an anaerobic chamber. After opening the caeca, the content was poured into a flask and diluted (1/10 w/v) in TGY broth (15% glycerol). The diluted floras were dispensed into polypropylene round bottom tubes and stocked in a deep freezer (-72°C) until use. In the same time, the flora was checked for the presence of *Campylobacter.*

In the second part of the experiment we tested, in four trials, the efficacy of the previously collected caecal floras and the MSC microflora (Wayne Farms LLC USA) to prevent *Campylobacter* colonization of chicks Isabrown (layer strain) reared in isolation units. The units are organized in several parts including a rearing area and a sas. Get in or get out of the birds, material needed for treatments or samplings, took place via a sas filled with quaternary ammonium compound. According to the trials three to five isolation units were used (one for the negative controls, one for the positive controls and one or more for the treated birds). Chicks delivered from the commercial hatchery are shared out amongst the isolation units (trial 1 : 184 birds – 5 units ; trial 2 : 76 birds – 3 units ; trial 3 : 99 birds – 4 units ; trial 4: 116 birds – 4 units). *Campylobacter* detection is realized on some birds sacrificed for analyze and by swabbings of the isolation units and transport crates.

As soon as the birds are placed into isolation units, every two days, until the birds were twenty days old, the caecal flora was administered to each treated bird by oral gavage of 0,1ml. MSC microflora was prepared according to the manufacture's instructions and 0, 2ml administered by gavage only the first day. Fifteen days after placement, all the birds, except negative controls were inoculated with a 0,1ml of a suspension of a *Campylobacter jejuni* strain isolated from an environmental sample of a rearing chicken house to provide about 10^2 cfu to each chick. One day before challenge and then 2, 6, 7, 12 or 16 days after challenge according to the trials, chicks from isolation units were sacrificed and the caeca removed aseptically for *Campylobacter* detection (Table I) . All the samples were diluted (1/10) into Preston broth and the swab tissues into 150 ml broth. Two selective media Butzler n°2 agar and Karmali agar were used for direct plating (100µl). Furthermore *Campylobacter* numeration was realized with a spiral plater system onto Karmali agar (incubation 48-72h, 42°C, microaerophilic conditions) . On the other hand an enrichment procedure in Preston broth was applied (18h-24h) before streaking on Butzler n°2 and Karmali agar.

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Results and discussion

Result expression (Stern *et al.* 2001)

Colonization rate : The colonization rate was calculated by dividing the number of birds colonized by the organism, by the number of birds challenged with the organism.

Colonization factor : Mean log $_{10}$ cfu *Campylobacter* per g in caeca of positive birds within each group. In our study we decided that the amount of *Campylobacter*/g of caeca was 100 when *Campylobacter* could be only detected after an enrichment period and 1 *Campylobacter*/g of caeca analyzed when they were not.

Protection factor : Ratio of mean log $_{10}$ of *Campylobacter* per g in caeca of positive control chicks to corresponding mean for treated birds.

Results

No *Campylobacter* could be detected from the caecal floras. Whatever the trials, before challenge, no *Campylobacter* was detected from the chicks delivered by the commercial hatchery and the negative controls remained negative all over the rearing period. The determination of the exact title of the different suspensions allowed the quantification of *Campylobacter* given to each bird . Trial 1 : 190cfu /chicks ; Trial 2 : 360cfu /chicks ; Trial 3 : 130cfu /chicks ; Trial 4 : 60cfu /chicks.

After challenge, *Campylobacter* colonized the positive controls in the first three trials (Table II). Two days after challenge the colonization rate is low but within 6 or 7 days the number of colonized birds increased and we noticed colonization factors ranging from 2,5 to 8,0. In the last trial, unexpectedly, challenge of 60cfu /chicks did not succeed to colonize the positive controls.

In the first trials, caecal floras collected in several occasions until the 14th day from the same laying hen flock were efficient in preventing colonization of the chicks Isabrown ; no *Campylobacter* could be detected in the caeca of the treated birds. MSC treatment administered in the third trial was not as satisfying because the colonization rates and the colonization factors observed were nearly the same and even more important than those of the positive controls : the protection factors are 1,0 and 0,5. In the last trial, treatment of the birds with caecal floras collected from another laying hen flock gave opposite results : on the one hand caecal flora (CFLBD24) can prevent colonization of the treated bird on the other hand caecal flora (CFLBD4) made colonization easier. In fact 12 days after challenge all the CFLBD4 treated chicks are colonized and the colonization factor is very high : it means 10,1.

Discussion

In three different occasions, challenge of 130, 190 or 360cfu/chicks resulted in *Campylobacter* colonization of the positive controls even if colonization was not as easy as that observed for broilers in other trials (Laisney *et al*. 2003). In a same way, Stern *et al* (1990) noticed that the resistance to caecal colonization was influenced through chicken host lineage. Caecal floras collected from one flock of young hen

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donors and administered to chicks “Isabrown” were able to prevent contamination of the birds ; the different batches of caecal floras giving the same result : no *Campylobacter* could be detected from the treated birds. The efficacy of these floras was perhaps enhanced by the fact that layers appeared to be less sensitive to *Campylobacter* colonization. Nevertheless, MSC microflora tested in the same time in the 3rd trial was not efficient . In fact the MSC product proved to protect chicks against salmonella colonization was less successful against campylobacter colonization (Stern *et al.*2001; Ferreira *et al.* 2003). Stern (1994) noticed that the efficacy of Mucosal Competitive Exclusion treatment decreased with storage of culture after 3 or 9 months of conservation in deep freezer. Our caecal floras from the first hen flock were efficient to prevent *Campylobacter* colonization of chicks Isabrown even after 20 months storage at -72°C. The reason why colonization of the positive controls did not occur in the last trial is unclear. The little amount of *Campylobacter* (60cfu) administered to the birds could be pointed out but, in the same trial, colonization took place for the birds treated with caecal flora collected the 4th day of the rearing period of another laying hen flock. Much more, twelve days after challenge the colonization factor of the CFLB4 treated birds is the more important we have ever seen previously. According to these observations, no comparison could be done between a young caecal flora (here 4 days) and an older caecal flora (24days). Another trial should be done using the same material and the same conditions. If caecal floras collected from the second laying hen flock are always inefficient in preventing colonization of chicks Isabrown it would be interesting to characterize, especially genotypically, the caecal floras collected from the first laying hen flock (efficient) and to compare them to the second one (inefficient).

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Table I : Number of caeca samples in isolation units according trials and days

Trial	Isolation unit	Number of days before and after challenge						
		-1	0	2	6	7	12	16
1	Negative controls	5		10	5		5	5
	Positive controls	5	challenge	10	10		4	4
	Treated CFLAD3	10	challenge	10	10		5	5
	Treated CFLAD7	10	challenge	10	11		5	5
	Treated CFLAD10	10	challenge	10	10		5	5
2	Negative controls	5		10	10			
	Positive controls	5	challenge	10	11			
	Treated CFLAD7	5	challenge	10	10			
3	Negative controls	5			10	10		
	Positive controls	4	challenge		10	10		
	Treated CFLAD14	5	challenge		10	10		
	Treated MSC	5	challenge		10	10		
4	Negative controls	5			12		12	
	Positive controls	5	challenge		12		12	
	Treated CFLBD4	5	challenge		12		12	
	Treated CFLBD24	5	challenge		12		12	

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Table II : Colonization Rates, Colonization Factors, Protection Factors according trials

Trial	Isolation unit	Number of days after challenge											
		2		6			7			12		16	
		CR	CF	CR	CF	**PF**	CR	CF	**PF**	CR	CF	CR	CF
1	Positive controls	0,1	0,5	1,0	6,5					1,0	2,5	1,0	6,5
	Treated CFLAD3	0,0		0,0						0,0		0,0	
	Treated CFLAD7	0,0		0,0						0,0		0,0	
	Treated CFLAD10	0,0		0,0						0,0		0,0	
2	Positive controls	0,0		1,0	8,0								
	Treated CFLAD7	0,0		0,0									
3	Positive controls			0,6	2,5		0,8	4,1					
	Treated CFLAD14			0,0			0,0						
	Treated MSC			0,5	2,6	**1,0**	1,0	8,2	**0,5**				
4	Positive controls			0,0						0,0			
	Treated CFLBD4			0,8	6,3					1,0	10,1		
	Treated CFLBD24			0,0							0,0		

CFALD3, CFLAD7, CFLAD10, CFLAD14: caecal flora collected from young hens (3, 7,10 and 14 days old) ; MSC : Mucosal Starter Culture ; CFLBD4, CFLBD24 : caecal flora from another batch of young hens (4 and 24 days old)

CR : number of colonized birds / number of challenged birds ; CF : mean $\log_{10}$ cfu *C.* /g caeca of positive birds ; PF : mean $\log_{10}$ cfu *C.*/g caeca of positive control birds / mean $\log_{10}$ cfu *C.*/g caeca of treated birds

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PREVENTION AND ELIMINATION OF *SALMONELLA ENTERITIDIS* IN POULTRY AND EGGS

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Keywords: *Salmonella enteritidis*, feed supplementation, egg yolk, poultry, shell eggs

Abstract

Salmonella enteritidis colonizes the chicken intestinal tract, contaminating shelleggs and contributing to human salmonellosis. In this study, a novel elimination and prevention method based on feed supplementation was investigated. Egg yolk powder and its protein components were tested. In the elimination study, the chickens were orally infected then given a supplemented feed of 5, 10 and 15% of each of the test samples. Fecal samples tested weekly showed an absence of *S. enteritidis* after the first week of feeding egg yolk powder and a gradual decrease with the yolk proteins. In the prevention study, Salmonella free chickens were fed the supplemented feed for 4 weeks then infected orally. Fecal samples tested for 4 weeks showed that *S. enteritidis* was prevented by egg yolk powder from colonizing the intestinal tract throughout the test period ; while the yolk proteins only delayed the colonization. Microflora counts in the intestinal tract were unaffected by any feed supplements.

Résumé

Salmonella Enteritidis (SE) contamine l'intestin des poules, les œufs et contribue ainsi aux salmonelloses humaines. Dans cette étude, une méthode d'élimination et de prévention des salmonelles par supplémentation alimentaire a été évaluée. De la poudre de jaune d'œuf et ses composants protéiques ont été testés. Des poulets inoculés par SE préalablement au traitement ont été nourris avec un aliment supplémenté à 5, 10 et 15 % des différents composants. Les échantillons de fientes sont devenus négatifs à la fin de la première semaine de traitement, avec de la poudre de jaune d'œuf, et la contamination des fientes a diminué progressivement chez les animaux supplémentés avec des protéines de jaune d'œuf. Des animaux nourris quatre semaines avec ces régimes, puis inoculés par SE n'ont pas été colonisés par ceux

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supplémentés en jaune d'œuf et ont vu leur colonisation retardée pour les autres. Les démembrements bactériens dans le tractus digestif n'ont pas évolué avec le traitement.

Introduction

The incidence of food-borne salmonellosis associated with *Salmonella enteritidis (S. enteritidis)*-infected eggs has increased in North America and Europe since 1979 (Snoeyenbos et al., 1996). Tranovarian transmission (Ryter, 1990; Shivaprusad *et al*., 1990) and penetration of the eggshell by *S. enteritidis* present in chicken feces deposited on the outside of the egg as it passes through the cloaca have been proposed routes for *S. enteritidis* egg contamination (Snoeyenbos et al., 1996)

The ability to adhere to and penetrate epithelial barriers is a common route of entry by *Salmonella* into poultry (Finlay et al., 1989; Moulder, 1985). Therefore, inhibiting adhesion may prevent colonization of the intestine by *S. enteritidis* and thereby eliminate and prevent the infection which is a major concern for both the poultry and food processing industries as well as for the consumer (Roberts et al.,1990). Hence, reducing intestinal colonization of *S. enteritidis* during the grow-out period is crucial to provide safer poultry meat and eggs, minimize economic losses, and reduce the spread of disease.

Feed supplemented with various components in an attempt to eliminate *S. enteritidis* from the intestine has been investigated for many years. D-mannose was reported to remarkebly suppress the colonization of *S. typhimuium in vitro* (McHan et al, 1989) and *in vivo* (Deloach, 1989); however, it is too expensive to be used in feed applications. Allen et al., (1997) has reported that palm kernel meal, which contains considerable amounts of structural carbohydrates such as arabinans, galactans, glucans, mannans, and pectins is effective in preventing *Salmonella* spp. infection; however, its preparation on an industrial scale has not been established, it may be difficult to put this into practical use. Furthermore, lactose was reported to be effective in the prevention of *S. typhimurium* infection (Corrier et al., 1990), but it has been suggested that the dosage of lactose used causes diarrhea and decreases the daily gain (Kogut, 1994). Another approach for the prevention of *S. enteritidis* colonization is provided by the administration of probiotic supplements (Fuller, 1990). However, this is still an expensive method and so used only for breeders and layers but not for broilers. Therefore, the purpose of this study was to search for a novel, practical, and effective method for the elimination and prevention of *S. enteritidis* infection in poultry using feed supplementation.

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Material and Methods

***S. enteritidis* isolate**-The isolate used for infection was *S. enteritidis* PT4 SA992212 from chickens supplied by Dr. Cornelius Poppe, Health Canada (Guelph, ON). 1 ml of 10^9 CFU/ml overnight culture adjusted by optical density at 660nm and 1ml of sterile tryptic soy broth (BD, Canada) as control were used for infection

Chickens- Twenty six 24 weeks old white leghorn hens (elimination study) and twenty hens (prevention study) were obtained from Arkell Poultry Research Unit, Guelph, Canada. Cloacal swabs and fecal samples were collected and tested for *S. enteritidis* by the preenrichment/enrichment selective plating method. Egg yolk samples were tested for antibodies to *S. enteritidis* by ELISA. All birds were negative for the aforementioned organism.

Housing- All birds were housed in separate wire-bottom cage systems at the Isolation Unit, University of Guelph, Canada. Feed was pelleted and contained no antibiotics. Plastic sheets were used under the wire floors of each cage to catch droppings

Experimental design- *Elimination study*: Feed and water were withheld for 24 hours prior to infection. Twelve chickens were inoculated orally with 1 ml of 10^9 CFU/ml of *S. enteritidis* and two with 1 ml of TSB broth using a syringe and a blunt-end catheter. A booster infection of another 1ml was given 1 week after the first infection. Three days post-inoculation, the infection was confirmed by testing cloacal swab specimens and fecal samples to monitor *S. enteritidis* level. Once the *S enteritidis* level in the feces reached 10^4-10^5 CFU/ml, supplemented feeding commenced. For 4 weeks, each four were fed respectively either a supplemented feed of egg yolk proteins or egg yolk powder at concentrations of 5%, 10% and 15%, and two control birds were fed the regular feed. *Prevention study*: For 4 weeks, each four *S. enteritidis* free chickens were given respectively a supplemented feed of 5 and10% egg yolk powder, 10 and 15% egg yolk proteins, and regular feed for the control. 4 weeks post-feeding, feed and water were withheld for 24 hours and all the chickens were orally infected with 1 ml of 10^9 cfu/ml of *S. enteritidis*. Post-infection, the chickens were fed the regular feed.

Bacteriologic examination-Fecal samples were collected once a week starting 4 days post-feeding (elimination study) or post-infection (prevention study) for 4 weeks. *S. enteritidis* was tested and enumerated as before. At the end of the experimental period, chickens were euthenized.

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Results and Discussion

Elimination study: Following infection, the average $\log_{10}$ levels of *S. enteritidis* in the fecal samples was 4.9 CFU/ml at week 2 (Figures 1a,b) At this level the supplemented feeding commenced and a decrease in the mean $\log_{10}$ levels of *S. enteritidis* counts were evident with 5, 10 and 15% egg yolk powder after 1 week of the feeding period (Figure 1a). A gradual decrease was detected with the 10 and 15% egg yolk proteins (Figure 1b) and and a slight decrease with 5 % yolk proteins.

Prevention study: Following the supplemented feeding period of 4 weeks, the chickens were infectedwith *S. enteritidis* and given the regular feed (Figures 2a,b). Fecal samples tested showed that *S. enteritidis* was not recovered from the chickens that were fed egg yolk powder supplement at all concentrations (Figure 2a) during the 4 week test period. The organism was also not detected for up to 2 weeks in the fecal samples of the chickens fed 10 and 15% egg yolk proteins, then the mean $\log_{10}$ levels *of S. enteritidis* increased to 4.7 CFU/ml at 4 weeks

Microflora: The mean $\log_{10}$ levels in the fecal samples of the total aerobic, total anaerobic, lactobacilli and enterobacteriacea counts remained constant throughout the infection and feeding period with a slight decrease in the enterobacteriacea from 4.0 CFU/ml to 3.0 CFU/ml as *S. enteritidis* levels decreased (Figure 3).

(1a) (1b)

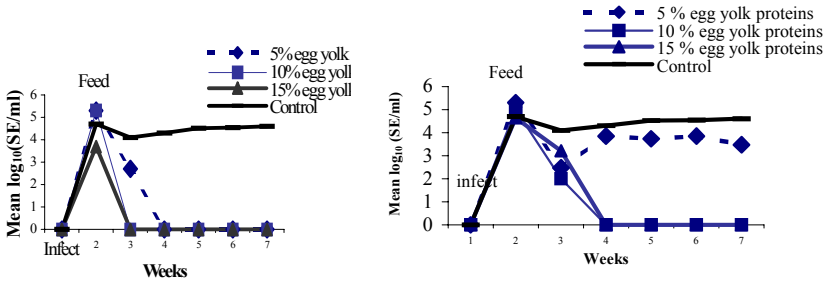

Figure 1 *S. enteritidis* counts in fecal samples when chickens were infected with *S. enteritidis* then fed for 4 weeks egg yolk powder and egg yolk proteins supplemented feed.

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(2a) (2b)

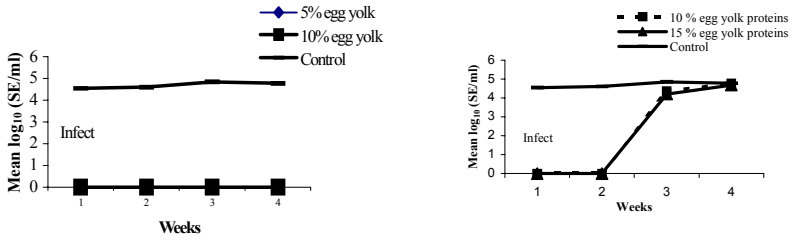

Figure 2. *S. enteritidis* counts in fecal samples after chickens were fed egg yolk powder and egg yolk protein supplemented feed for 4 weeks then infected with *S. enteritidis*.

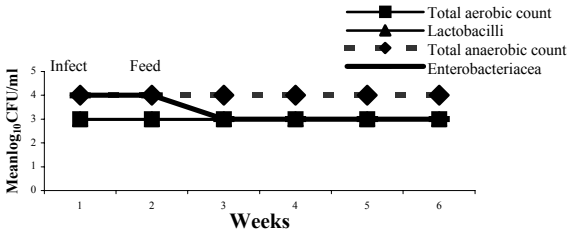

Figure 3. Total microflora counts in fecal samples throughout the study.

In conclusion, *S. enteritidis* colonizes the intestinal tract of poultry and causes food borne illness in humans; therefore, the elimination and the prevention of this organism from laying hens, broilers and other poultry before they reach the processing plant will improve the chances of producing processed carcasses free from these organisms. (Stern et al., 2001). Because the adhesion of this bacteria in the intestinal epithelial cells is crucial to the initial phase of infection (Ofek et al., 1994), blocking the adhesion in the intestine may prevent infections. Hence, egg yolk powder and its protein components were investigated in this study as a potential solution. Konishi *et al*, (2002) reported that egg yolk derived sialyloligosaccharide and sialylglycopeptide have anti-bacterial properties and provide protection against gastric diseases such as salomonella infection by preventing bacteria from binding to the intestine rather than by activating macrophages.

From the present results,, egg yolk powder was able to both eliminate and prevent *S. enteritidis* intestinal colonization at a concentration as low as 5%, in addition, egg yolk powder can be readily available, practical and economic. Egg yolk proteins did show an elimination effect, this suggested that the proteins in the egg yolk play a role in the

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elimination and prevention but other components present in the egg yolk also contribute to this activity probably by a synergistic effect. To determine the functional components in the egg yolk as well as the possibility of any synergistic effect, concurrent esperiments are being conducted using a cell culture model system.
Furthermore, the feed supplements must not disrupt the natural microflora in the chicken's intestine. Thus, fecal samples tested throughout the study, showed that none of the fed supplements at any concentration had a negative effect on the intestinal microflora of the chickens, the counts remained constant except for the enterobacteriacea as the level slightly decreased after *S .enterittidis* was eliminated from the intestine as it is counted under this group.

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MONITORING CHORINATED HYDROCARBON RESIDUES IN POULTRY

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Keywords: chlorinated hydrocarbons, chlordane, DDT, dieldrin

Abstract

Environmentally persistent contaminants from past industrial and agricultural practices, and chemicals released into the environment from present-day practices appearing as residues in edible tissues of poultry at time of slaughter can result in condemnation of the product and, if passed onto the consumer, will lower their confidence in the safety of the poultry supply. Results from experiments indicate that chlorinated hydrocarbon (CHC) residues such as DDT, chlordane, and dieldrin are assimilated into the uropygial gland (UG) and it is possible to monitor these residues in the live bird. Results indicate that measurement of CHC residues in UG fluid collected from the live bird could be used as a viable alternative to collection of abdominal fat for preslaughter residue testing in broilers and turkeys.

Resume

Les contaminants environnementaux passés et présents peuvent s'accumuler dans les tissus des volailles et leur présence dans la viande peut entraîner une saisie du produit et une perte de confiance des consommateurs. Les résultats de cette expérimentation montrent que les carbohydrates chlorés comme le DDT, le chlorane et la dieldrine sont stockés dans la glande uropygiale (UG) et qu'il est possible de les doser sur les oiseaux vivants. Les résultats montrent que cette méthode de mesure in vivo peut être une alternative intéressante au dosage traditionnel sur le gras abdominal chez les poulets et les dindes.

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Introduction

Poultry can be exposed to chemical or biological products from a variety of sources such as medications, pesticides, equipment, or even building materials. Numerous environmentally persistent contaminants resulting from past industrial and agricultural practices, and chemicals released into the environment from present-day practices may appear as residues in edible tissues of poultry at time of slaughter. Drug or chemical residues remaining in poultry at time of slaughter can result in condemnation of the product and, if passed onto the consumer, will lower their confidence in the safety or wholesomeness of the poultry supply. A vigilant residue prevention program is essential to fostering the prudent use of drugs and pesticides in animals that enter the human food supply. Preventing residue at all stages of animal production is a responsibility that must be shared by all segments of the industry. In the United States, the Food Safety and Inspection Service (FSIS) National Residue Program (NRP) provides a variety of sampling plans to verify and enforce that slaughter establishments are fulfilling their responsibilities under the Hazard Analysis and Critical Control Point (HACCP) regulation, and in accordance with Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) regulations, to prevent the occurrence of violative residues. The purpose of the NRP is to identify the critical points in animal production for residue prevention and to educate producers to adopt controls, which ensure that marketed food animals do not contain illegal residues.

For example, in a voluntary residue monitoring program for poultry, four to ten birds per flock are sacrificed 5-10 days prior to slaughter and samples of abdominal fat (AF) are submitted for analysis of chlorinated hydrocarbon compounds (CHCs). Types of CHCs typically screened during a typical analysis include: lindane, chlordane, dieldrin and aldrin, DDT and metabolite, endrin, heptachlor and epoxides, polybrominated biphenyl (PBB), methoxychlor, mirex, toxaphene, polychlorinated biphenyl (PCB), hexachlorobenzene (HCB), and chlorpyrifos.

Various names have been applied to the uropygial gland: examples are oil sac gland, preen gland, and rump gland. The most exact name would be *glans uropygii*. The uropygial gland (UG) is a bilobed organ embedded beneath the dorsal side of the base of the tail. Its sole function is to secrete fatty acid esters during the act of «preening» for maintenance of water repellent quality and structure of feathers. The structure and function of the UG has been reviewed (Lucas and Stettenheim, 1972).

There are few reports indicating that uropygial gland fluid (UGF) can be successfully collected from live birds (Larsson and Lindegren, 1987). The lipid composition of UGF may potentially be utilized to monitor lipophilic residues (i.e. chlorinated hydrocarbons) (Apandi and Edwards, 1964; Tang and Hansen, 1976; Jacob, 1978). If a method were developed to monitor residues in the live bird by collection of UGF,

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then the levels of residues may be determined using a more proficient sampling method without sacrificing the animal.

Materials and Methods

Collection of Uropygial Gland Fluid

Inadequate amounts of UGF were obtained when fluid was expressed from the gland, and contamination was encountered with this method. Withdrawing fluid from the gland utilizing a 3 ml syringe fitted with a 3.5 cm x 18 gauge needle yields approximately 0.3 ml or 0.1 ml of clean UGF from a single turkey or broiler, respectively. Insertion of the needle below and to the outside of each lobe yields the best results and successful collection results in a slight pucker of the skin as the fluid is being withdrawn. Fluid should be expelled from the syringe immediately after collection, since UGF solidifies rapidly once removed from body temperature.

Broiler Experiment

In an experiment with broilers, twelve 37 day-old broilers were divided into two groups (six birds/group) and administered one of two levels of DDT (15 or 30 mg) and dieldrin (1 or 3 mg) in a single encapsulated dose. At 42 and 46 days of age, UGF was obtained from three birds/treatment. Birds were then sacrificed and a sample of abdominal fat (AF) was obtained for analysis.

Turkey Experiment

Thirty-two 18-week-old Large White male turkeys were divided into four groups (eight birds/group). Four birds per group were administered either (1) chlordane alone or (2) dieldrin and DDT in three encapsulated doses over a period of six days. Each group was administered one of four levels of the respective compounds. Chlordane was administered to four birds per group amounting to a total dose of 3, 6, 9 or 12 mg/bird. Dieldrin and DDT was administered to the remaining four birds per group amounting to a total dose of 3, 6, 9, or 12 mg/bird for dieldrin and 60, 105, 150 or 225 mg/bird for DDT. Seven and 14 days after administration, UGF was obtained from two birds per dosage level. The birds were then sacrificed and a sample of AF was obtained.

Samples of UGF and AF were analyzed according to AOAC methods (AOAC, 1984) specified in Chemistry Quality Assurance Handbook (USDA, 1982).

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Results and Discussion

Broiler Experiment

For dieldrin, the higher dose level produced differences between UGF and AF, whereas levels of DDT in AF were typically higher than levels found in UGF (Figure 1). Results from this study indicate that UGF can be successfully collected from the broiler and monitored for CHC residues, although the residues may be assimilated into the AF at a higher rate and conversely eliminated from UGF more rapidly. Such information may suggest a need to establish different action levels when using UGF for monitoring CHC residues.

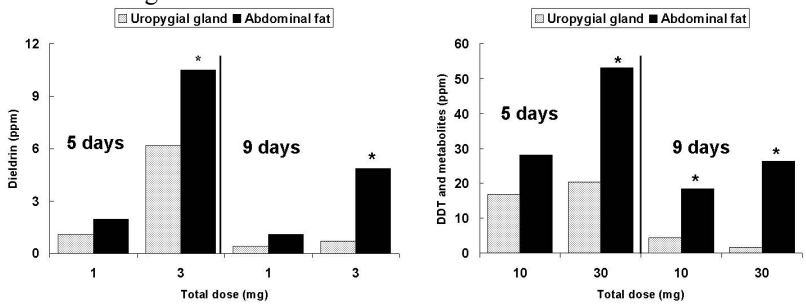

Figure 1. Amount of dieldrin and DDT (including DDE and DDD) in uropygial gland fluid (UGF) and abdominal fat (AF) of the broiler chicken when administered a single dose of these compounds at 37 days of age and subsequently sampled at 42 and 46 days of age. Asterick indicates significant differences (P<.05) between UGF and AF.

Turkey Experiment

Results indicate no differences in the amount of chlordane in UGF or AF for the 2-week period following administration (Figure 2). Dieldrin level differed between the two tissue types at the 12 mg dose level 14 days post administration. Significant differences between the amount of DDT in UGF and AF occurred predominantly when higher levels were administered. It appears that DDT is not as readily assimilated into UGF as compared to AF.

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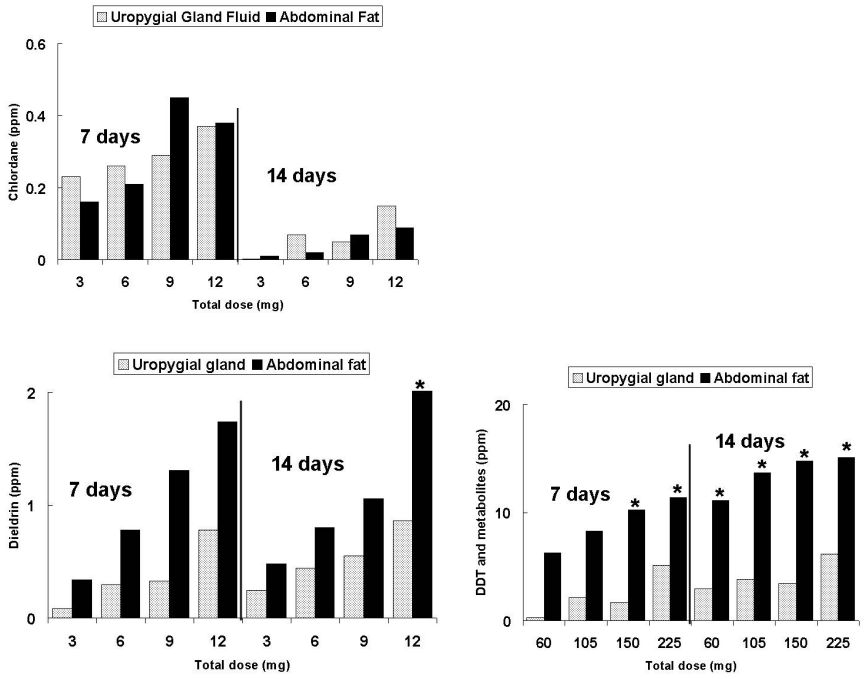

Figure 2. Amount of chlordane, dieldrin, and DDT (including DDD and DDE) in uropygial gland fluid (UGF) and abdominal fat (AF) of the Large White male turkey when administered three equal dose of these compounds over a six-day period and sampled at seven and fourteen days following administration. Asterick indicates significant differences (P<.05) between UGF and AF.

Results from several experiments with broilers and turkeys indicate that CHCs such as chlordane, dieldrin, and DDT are assimilated into UGF and it is possible to monitor these compounds in the live bird by the collection and analysis of its UGF. The measurement of CHC residues in UGF collected from the live bird is a viable alternative to sacrifice and collection of AF. The levels of CHC residues detected in UGF may be somewhat lower in comparison to AF depending on age, amount, and type of compound consumed for the collection and analysis of UGF for CHC residues. The intent of these studies were to provide a dosage level that would result in abdominal fat residue levels about the EPA established action levels of 0.3, 0.3, and 5.0 ppm for chlordane, dieldrin, and DDT, respectively. Results from both studies resulted in tissue levels above these established action levels.

The applicability of this method is not just restricted to broilers and turkeys. This method may also be useful to other species of poultry (ducks, geese) as well as wild

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avian species where valuable specimens can be monitored without sacrifice. Therefore, the collection of UGF may be useful in determining the presence of CHCs for a wide spectrum of avian species in a simple and ethically attractive way.

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CONTENTS

MICROBIOLOGICAL QUALITY OF PRESSURE-TREATED POULTRY PRODUCTS

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Keywords: high pressure processing, poultry meat, egg products, safety, shelf-life

Abstract

High pressure processing is applied to several poultry products for safety enhancement and shelf-life extension. Pressurization induced large decreases in counts of psychrotrophic, lactic acid bacteria, and especially enterobacteria and *Listeria* spp. Reductions were similar to or even higher than those caused by heat treatment. High pressure and nisin, pressurization at 50 ºC, and cycle pressure treatments effectively inactivated indigenous and inoculated microbiotas (e.g., *Escherichia coli* and *Salmonella* Enteritidis). High pressure processing greatly improves the microbiological quality of poultry products.

Résumé

L'application des hautes pressions sur plusieurs produits de volaille améliore la sécurité et prolonge la durée de vie. Les hautes pressions provoquent d'importantes réductions du nombre des psychrotrophes, des bactéries lactiques mais surtout des entérobactéries et de *Listeria* spp, les réductions étant similaires ou même supérieures à celles obtenues pendant les traitements thermiques. On obtient l'inactivation effective des microorganismes naturellement présents ou inoculés (e.g., *Escherichia coli* et *Salmonella* Enteritidis) avec quelques uns des traitements suivants: haute pression avec de la nisine, haute pression à 50 °C ou haute pression par cycles. Les hautes pressions améliorent d'une façon importante la qualité microbiologique des produits de volaille.

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Introduction

General aspects

High pressure processing is the technology by which a product is treated at or above 100 MPa. Pressure is transmitted uniformly and almost instantaneously throughout the food, regardless of its size and shape. Furthermore, once the pressure value is reached, there is no need for extra energy. Pressurization can be performed at temperatures lower than those used with heat treatment. Thus, the nutrient content and some organoleptic characteristics, such as odour and taste, do not markedly change.

Microbial sensitivity

Indigenous microbiota grows more readily than inoculated laboratory collection strains. Gram-positive bacteria are more resistant than Gram-negative bacteria, and cocci are more resistant than rods. Bacterial spores are the most pressure-resistant microorganisms. Cells in the logarithmic growth phase are more sensitive than those in the other phases.

Treatment effectiveness

Increasing pressure and exposure time lead to increased lethality. Treatments consisting of several cycles are generally more effective than continuous treatments, especially against bacterial spores. The effectiveness of pressurization is greater in inoculated suspensions and model systems than in foods. The lower the pH and the higher the water activity, the more lethal the treatment.

Objective

To investigate pressure inactivation of pathogenic and spoilage bacteria in poultry products for safety enhancement and shelf-life extension, with particular emphasis on techniques to detect sublethally injured cells.

Materials and methods

Raw materials

Mechanically recovered poultry meat, cooked poultry sausages, whole and minced chicken (breast and thigh), liquid whole egg, egg yolk and white.

High pressure processing

Combinations of several pressures (100 to 900 MPa), times (1 to 30 min, continuous and cycle pressurizations) and temperatures (−20 to 75 ºC). With two discontinuous isostatic presses: one, with a *ca.* 2-L volume pressure vessel, from ALSTOM (Nantes, France) for 100- to 500-MPa treatments; and the other one, with a *ca.* 275-mL volume pressure vessel, from Standsted Fluid Power (Standsted, UK) for 600- to 900-MPa treatments. Treatment temperature monitored with a thermocouple. Samples allowed

to reach the treatment temperature inside the pressure vessel before pressurization. The effect of pressure treatment usually compared with that of conventional heat treatment.

Microbiological analysis

Counts of indigenous and inoculated pathogenic (*Clostridium perfringens*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Staphylococcus aureus*, *Yersinia enterocolitica*) and spoilage bacteria (lactic acid bacteria, enterobacteria, total/faecal coliforms, sulfite-reducing clostridia), on nonselective and selective media. Techniques to detect and recover sublethally injured cells.

Results

The following are some particular results.

Mechanically recovered poultry meat (Table 1)

Mechanically recovered poultry meat had high initial counts (7 log CFU/g) and reached 9 log CFU/g at 15 days. Increased inactivation occurred with increasing magnitude of pressure. Psychrotrophs were more pressure-sensitive than mesophiles. Combination of high pressure and 200 ppm nisin plus 1 % GdL was very effective: psychrotroph counts of mechanically recovered poultry meat treated at 450 MPa were less than 1 log CFU/g throughout the 30-day experiment.

TABLE 1. Counts (log CFU/g) of mechanically recovered poultry meat pressurized at 2 ºC for 3 cycles of 5 min each, and stored at 2 ºC.

			Day 1	Day 15	Day 30
No additives	Mesophiles	Untreated	6.92	9.04	9.12
		350 MPa	5.56	6.86	8.22
		450 MPa	5.06	5.35	5.91
	Psychrotrophs	Untreated	7.01	9.09	9.22
		350 MPa	2.88	4.89	8.22
		450 MPa	2.77	3.04	5.82
Nisin + GdL	Mesophiles	Untreated	5.19	8.27	8.83
		350 MPa	2.54	2.47	2.90
		450 MPa	2.47	1.41	1.50
	Psychrotrophs	Untreated	5.15	8.31	8.79
		350 MPa	1.37	1.34	2.79
		450 MPa	0.65	0.95	0.88

GdL, glucono-delta lactone.

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Cooked poultry sausages (Table 2)

Cooked poultry sausages had high initial counts and, at 6 weeks, reached *ca.* 8 log CFU/g of lactic acid bacteria, 4 log CFU/g of *Listeria* spp. and 5.5 log CFU/g of enterobacteria. High pressure processing was more effective against lactic acid bacteria and *Listeria* spp. than heat processing. Both treatments were very effective against enterobacteria. No *Listeria* spp. and enterobacteria growth occurred in pressurized sausages throughout the 18-week experiment.

TABLE 2. Counts (log CFU/g) of cooked poultry sausages pressurized at 500 MPa at 65 ºC or heat-treated at 80 to 85 ºC for 40 min, and stored at 2 ºC.

		Day 1	Week 3	Week 6	Week 9	Week 12	Week 15	Week 18
Lactic acid	U	4.05	6.06	7.74	7.90	7.98	8.20	8.15
bacteria	P5	0.35	n.d.	0.48	0.98	0.50	0.93	n.d.
	P15	0.35	n.d.	0.30	n.d.	0.68	0.30	n.d.
	H	2.01	1.39	2.07	1.97	1.48	1.99	1.40
Listeria spp.	U	2.13	3.33	4.10	4.27	4.44	4.44	4.60
	P5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	P15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	H	1.59	1.00	1.33	1.00	1.33	1.00	1.00
Enterobacteria	U	3.51	4.64	5.38	6.02	5.62	5.23	5.01
	P5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	P15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	H	n.d.	n.d.	0.35	n.d.	n.d.	n.d.	n.d.

U, untreated; P5, pressurized for 5 min; P15, pressurized for 15 min; H, heat-treated; n.d., not detected.

Liquid whole egg (Tables 3 and 4)

Listeria innocua was more pressure-resistant than *E. coli*. Furthermore, since *Listeria* is a psychrotrophic organism, surviving (injured) *L. innocua* cells recovered and developed and counts increased during the refrigeration storage. In contrast, surviving (injured) *E. coli* cells died and counts decreased, because *E. coli* is not psychrotrophic. *Listeria innocua* was more sensitive to high pressure-nisin combination than *E. coli*: *L. innocua* was not detected throughout the 30-day experiment. This is because nisin is more effective against Gram-positive bacteria.

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TABLE 3. Counts (log CFU/mL) of inoculated liquid whole egg, with or without nisin (5 ppm), pressurized at 450 MPa for 10 min at 20 ºC, and stored at 4 ºC.

		Day 1	Day 8	Day 15	Day 30
E. coli	Untreated	5.97	6.51	6.27	6.27
	HPP	2.35	1.82	1.10	n.d.
	HPP + nisin	1.56	1.09	n.d.	n.d.
L. innocua	Untreated	6.43	7.14	7.53	7.36
	HPP	4.93	5.02	4.82	6.08
	HPP + nisin	n.d.	n.d.	n.d.	n.d.

HPP, high pressure processing; n.d., not detected.

Salmonella Enteritidis was more pressure-sensitive than *E. coli*. Treatments at 50 ºC were the most effective and those at 2 ºC were the least effective. Cycle pressurization was more effective than continuous pressurization, which was very clear in liquid whole egg treated at 20 ºC.

TABLE 4. Counts (log CFU/mL) of inoculated (at *ca.* 7 log CFU/mL) liquid whole egg pressurized at 450 MPa for several continuous and cycle times at several temperatures.

	ºC / min	10	5 + 5	15	5 + 5 + 5
E. coli	2	4.19	3.79	3.55	3.13
	20	3.73	n.d.	2.92	n.d.
	50	n.d.	n.d.	n.d.	n.d.
Salmonella	2	3.86	3.18	3.32	3.86
Enteritidis	20	2.16	n.d.	1.47	n.d.
	50	n.d.	n.d.	n.d.	n.d.

n.d., not detected.

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EGG CONSUMPTION PATTERNS AND RISK ASSESSMENT ON *SALMONELLA*

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Keywords : Egg consumption, risk assessment, *Salmonella*

Abstract

Eggs are important vehicles of *Salmonella*, a leading cause of foodborne illness in many countries. In Finland, many risk management options are in place to combat this risk. In order to estimate the consumer risk of obtaining *Salmonella* infection via eggs and to evaluate preventive tools, a risk assessment will be built. However, data on consumer behaviour and egg consumption patterns are lacking. Therefore, egg use, purchasing behaviour, storage time, and storage temperatures in Finnish households were studied by a national survey in spring 2003. A random sample involving 3,000 adults was taken from the population register. Suitability of the Internet as a modern survey method was compared with the traditional postal survey by a questionaire with 40 questions. Results will be utilized to reveal proportions of low, medium, and high egg consumption patterns and to evaluate probability distributions of storage time and storage temperature of eggs in households. In addition, the two different survey methods will be compared.

Résumé

Les œufs sont une source de salmonelle et une cause majeure de toxi-infections alimentaires dans de nombreux pays. La Finlande a mis en place de nombreuses mesures de maîtrise pour lutter contre ce risque. Cette analyse a été conduite afin d'évaluer le risque, pour le consommateur, d'être contaminé par la salmonelle lors de la consommation d'un œuf. Pourtant, les résultats sur le comportement des consommateurs et leur consommation d'œufs manquent. En conséquence, une enquête sur l'utilisation des œufs, les comportements d'achats, les temps et températures de stockage dans les ménages finlandais a été étudiée au cours du printemps 2003. 3000 adultes ont été interrogés après tirage au sort. Les techniques d'enquête par internet

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versus une enquête postale traditionnelle (40 questions) ont été comparées. Les résultats seront utilisés pour définir les profiles de consommateurs faiblement, moyennement et fortement consommateurs d'œufs et pour évaluer la distribution des conditions de stockage dans les foyers. Ces deux méthodes d'enquête seront comparées.

Introduction

Salmonellosis caused by the ingestion of foods containing *Salmonella* bacteria is a major problem in many countries worldwide. Since the mid 1980s, there has been a dramatic increase of *Salmonella* outbreaks caused by *S.* Enteritidis both in the USA and in Europe (Humphrey, 2000). In 2001, salmonellosis and campylobacteriosis were the most frequently reported zoonooses in Europe, with approximately 159,000 cases each (European Commission, 2003). One of the most common food sources for these outbreaks have been shell eggs and egg-containing food products. In order to estimate the consumer risk of obtaining *Salmonella* infection via eggs and to evaluate different preventive tools, several risk assessments can be built (USDA-FSIS, 1998; WHO-FAO, 2002). An important piece of information required for these assessments is the consumer behaviour, which determines the storage conditions of eggs and consumption patterns of different egg dishes. Unfortunately, these data are often scarce. A traditional method to gather this type of information is a postal enquiry. Nowadays Internet offers a fast alternative to contact people. The purpose of the present study was to survey how Finnish households purchase, store and use eggs and to compare two different survey methods. The data will be used for building a national risk assessment on *Salmonella* in egg production.

Materials and Methods

A random sample of Finnish adults in the age groups from 15 or older was selected from the National Population Register. The sample size was 3,000. The respondents were devided into three equal-sized categories. The first group (Group A) received a letter asking them to fill the questionaire via Internet. The second group (Group B) received a questionaire form, but they were also told that it was possible to participate via Internet. The third group (Group C) only received the questionaire form. All respondents were told that it would take approximately 10 minutes to complete the enquiry. Letters in Finnish were mailed in May, 2003.

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The questionaire contained 41 questions. The questions were asked about purchasing behaviour of a family as well as storage times and storage conditions of eggs. Several questions were asked about different types of egg dishes prepared with the average using frequency, typical amounts used and a typical number of egg users within a family. Basic background sosiological information was also asked. All answers were first processed with the Webropol Realtime Analyzer software, which was used for producing the electric questionaire form in Internet. It also gave preliminary distributions of the data. The statistical analysis will be done using SPSS statistical program Version 11.5.

Results and Discussion

The overall response rate for the survey was 28%, and the preliminary results are presented here. The response rate for the traditional postal survey was clearly better than for the Internet survey. Only 10% of those who were asked to answer via Internet (Group A) filled in the electric questionaire. Accordinly, only nine persons from the thousand who were asked to participate using either mail or Internet (Group B), choose Internet. On the contrary, 348 persons returned the postal questionaire in the group B. In the group C, the response rate was 37%. Persons who answered via Internet were younger and they had better education compared with the other respondents. Internet as a survey tool gave quick results because the tedious data input step was not necessary. On the other hand, the respondents did not represent the whole population and the response rate was low. As a result, the Internet survey did not appear as a suitable tool for a large data collection in normal, unselected population.

One percetage of the all respondents reported that they did not use eggs at all. Around 10% told using eggs almost every day. The most people said they used eggs once or twice in a week. The most respondents (over 50%) bought eggs at least once every second week. Over 80% bought packed eggs in a grocery. Packing of 10 eggs was the most common number of eggs people usually purchased at a time. Around 90% of the respondents stored eggs in the refrigerator or in a cool storage. One fifth reported that they did not use eggs after their best before datum. On the other hand, over 50% said that they sometimes or often use eggs after their best before datum.

The most frequently prepared egg dishes were low risk foods containing well cooked eggs. While such foods themselves won't be risky, one should not forget the possibility of the cross contamination during the preparation of the dish. For instance, over 40% of the respondents said that they did not wash their hands at all after handling of eggs or did so only if it was necessary. From the egg dishes containing lightly prepared eggs, soft boiled eggs and fried eggs sunny-side up were mentioned most often. The raw eggs were consumed infrequently. The most common habbit was

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to taste different homemade batters. While the general trend was not to eat raw eggs, there were a tiny percetage of the respondents who reported the regular use of either raw eggs as such or egg dishes containing raw eggs. Our results agree with Grijspeerdt et al. (1999), who surveyed egg consumption of parents of Belgian college students. They find out that 98.2% of the consumption patterns fall in the low risk category, while only 0.9% of the cases had high risk behaviour. The present results will be further used in modelling the consumption step in the *Salmonella* risk assessment on eggs. Finally, the primary production model (Ranta and Maijala, 2002), already demonstrated for broilers, will be modified and connected with the present consumption data in order to obtain a model from farm to table.

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FOOD SECURITY PROGRAM DEVELOPMENT AS PART OF COMPREHENSIVE FOOD SAFETY PROGRAMS

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Keywords: Food Security, Food Safety

Abstract

Significant threats to the food production system, including potential terrorist attacks have necessitated the development of new strategies as part of comprehensive food safety/food security programs. A new program, Security Analysis and Risk Assessment Program (SARAP) was developed to meet these needs. The SARAP Program concept provides an organized methodology, by which vulnerabilities within the meat and poultry processing environment can be identified, testing and validation standards established and product quality and safety standards met. Once adopted, SARAP will better ensure the continued availability of a safe, readily available and economical food supply.

Résumé

Les nouvelles peurs alimentaires, incluant celles liées au bioterrorisme, ont nécessité une nouvelle approche stratégique des programmes d'assurance de la sécurité des aliments. Un nouveau programme d'analyse des risques et de la sécurité des aliments (SARAP) a été développé dans ce but. Le concept du programme SARAP fournit une méthodologie organisationnelle, qui permet d'identifier la vulnérabilité d'une production, de valider les critères applicables à cette production et de s'assurer qu'il sont atteints. Une fois mis en place, le concept SARAP assure la disponibilité continue d'aliments sûrs et économiques.

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Introduction

Recent events have made clear the potential threat from terrorist activities. The Public Health Security and Bioterrorism Preparedness and Response Act (2002) gives the Food and Drug Administration (FDA) in the United States (US) the authority for protecting the nation's food supply against terrorist acts and other threats. The Office of Food Security and Emergency Preparedness within the Food Safety and Inspection Service, USDA (2003) similarly monitors food security efforts within the meat and poultry sector of the food industry.

Agriculture everywhere must be considered to be a national security asset or "critical infrastructure", without which society could not function. Once identified as such, a new paradigm must be developed where resources are made available to assess the risks, identify the vulnerabilities and better protect the integrity of the food production system. Within this context, critical points within the production system, vulnerable to adulteration, must be identified and methodologies developed to negate the risks. Each phase of production and processing chain must be likewise surveyed and solutions found.

Material and Methods

In an effort to develop tools by which food processing plants could better assess their security vulnerabilities, the concept of a Security Analysis and Risk Assessment Program (SARAP) was developed. First discussed by Norton (2002), SARAP is a methodology, structured in a similar fashion to that of the Hazard Analysis and Critical Control Point (HACCP) system (ICMSF, 1988 ; Bauman, 1990), which provides a systematic approach, whereby critical security control points (CSCP) are identified. CSCPs are considered those points in the processing system, where adulteration could most likely occur. Once identified, they can be altered or the procees modified, reducing the level of vulnerability.

SARAP plan development begins long before the live bird or poultry product (egg, egg product, deboned meat, etc.) arrives at the plant. Each level of security from the farm to the eventual warehouse and transportation system to the consumer is critical, since the overall strength of the program is only achieved when each layer of the system provides adequate safeguards. Identification of the inputs at the processing plant is the first goal, since product adulteration can easily occur here. Often the points of entry into the plant are the most chaotic and least frequently monitored. Ingredient supplies are especially critical and should be considered high vulnerability items, since low levels of contamination can rapidly fan out from these as they are

dispersed in the process. At a minimum, all ingredients should be certified as to purity upon arrival.

Input points also considered in SARAP programs and therefore constantly monitored include personnel entrances, air and water intake systems and packaging material loading docks. Chemicals not directly used in or on the food products, including anti-microbial treatments and plant sanitation products must also be considered vulnerable to adulteration and therefore monitored and protected, since They too could be used to indirectly contaminate food products. Other less obvious critical input points include, water sanitizing and ice production systems. Some of these inputs may be covered under the HACCP plans and unique to each facility.

An important consideration in any SARAP program is the threat offered by employees. Throughout the world, examples can be cited where disgruntled or politically motivated employees were found to be responsible for product adulteration and contamination. In a recent case in the US, processed hams were recalled for adulteration due to physical hazards (screws and other hardware), which had been intentionally placed by an employee.

Once inside the plant, the product must be kept under constant scrutiny. Points where products become concentrated should be considered especially vulnerable, since these are the easiest sites of employee sabotage. Unfortunately, due to the complexity of design of the modern food processing plant, these critical concentration points are often neither easily accessible or monitored. The placement of continuously operating electronic surveillance equipment and implementation of retricted entrance into high-risk areas is often the easiest and most cost effective solution.

Following the identication of critical entry points, the next step in SARAP plan development is to establish critical security control solutions at each of these points. Critical security limits must be set for each of these security points, meaning that contingency plans must be established, monitored and maintained. An example of a security limit would be a concentration point in a processing plant where a surveillance camera is located. Should the camera fail, the critical security limit would establish that further product would not be moved into the concentration point, until the camera was again operational. Product already within the concentration point when the failure occurred could also be required to be segregated, examined and tested before it was moved further within the system.

Critical limits should, at a minimum, be designed to ensure maximized acceptable reduction of risk, which must be determined with consideration of litigation potential and input from other potentially affected stakeholders including, insurance carriers, governmental regulatory and security agencies, as well as the retail end users. Although, all company decisions must consider the effect on profit, SARAP and other security plans should be considered an investment in brand quality and financial integrity maintenance.

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Critical security limits also include the need for validated sampling strategies, designed to detect adulteration at the sensitivity levels established by regulation or internally within the company. Various sampling methods exist (ICMSF, 1986) to set limits, so that each lot of finished product can be sampled for a given potential adulterant or biological agent. A mechanism should be established by which the samples are taken a prescribed number of times. A validated method may be as simple as someone actually counting the samples, or as sophisticated as the installation of a calibrated machine that serves the same function. Should the lot be sampled an insufficient number of times, the prescribed limit would prevent the lot from moving further though the system.

Critical limits must also be accompanied by a prescribed solution. When missing a test, the prescribed soultion may be that the lot would be segregated in a portion of the warehouse, where it could not comingle with product meeting the established testing standards. Once the product was examined or required testing completed in the segregation area, if appropriate, the product could be moved back into the system.

Using another type of strategy, multiple types of tests might be required (eg. A, B, and C). Whereas, A and B might involve direct food safety tests, test C might involve testing an entire lot for the presence of a chemical adulterant. An example would be that test A and B consist of assays for *Salmonella spp.* and *Campylobacter* spp. taken from the meat products, while test C could involve an test for airborne contamination in the shipping containers. If all were completed, the lot of product could then be shipped ; if not, shipment would not proceed.

Sampling should never be made so predictable that a casual observer could discern a pattern, which could then be circumvented. If samples were to be routinely taken and tested only when food item number 10,000 passes a critical security point in the system, there remains 9,999 other opportunities for adulteration to occur, prior to the next testing. This problem is easily solved by providing a statistically valid, random testing scheme, whereby the actual sampling strategy is known only to the SARAP or HACCP management team. If applicable requirements for both HACCP sampling and SARAP sampling can be accomplised simultaneously, testing and validation should also operate in tandom, preventing the need for redundant personnel and duplication of effort. A useful and inexpensive methodology for both sampling schemes (HACCP and SARAP) is to determine when samples should be taken through the use of a random number generator, a common feature on most scientific calculators. The use of this prevents the introduction of a discernable pattern, which could be circumvented by a malevolent observor, but also has the added benefit of providing a more statistically valid approach, preventing the introduction of bias (intentional or unintentional) into the sampling scheme.

The next phase of planning is to prescribe corrective actions. As noted in the previous testing example, if a set of tests were not completed, the system must be designed to segregate the product, so that it cannot be comingled with product that has met the

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SARAP standards. To accomplish this, space must be made available for storing the product and clear instructions must be developed and made readily available to those personnel that would be involved.
Within the requirements of all phases of a fully functional SARAP program is the need for methodical record keeping. The actual requirements for records will vary according to the jurisdictional requirements, but could include such items as laboratory notebooks containing sampling results, surveillance tapes, equipment readout recordings or documented observations. It is important that all of these records be treated as legal documents, since they may be critical features for protecting against liability. Records must also be kept and regularly reviewed, since they can serve as an important source of information including trends and incidents, by which predictions might be formulated.

Results and discussion

Animal agriculture and food processing requirements continue to evolve with changes in regulatory requirements. All comprehensive food safety programs will eventually have to incorporate strategies for dealing with the issue of food security. The SARAP Program concept is an example of an organized methodology, by which vulnerabilities within the meat and poultry processing environment can be identified, testing and validation standards set and product quality and safety better assured. Combined, with other similar security programs in other phases of live animal production, product warehousing and transportation, SARAP begins to establish a comprehensive protective umbrella, by which the continued availability of a safe, secure and economic food supply can better be assured.

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Table 1. Minimum requirements for Security Analysis and Risk Assessment Program Development

1. List the potential food product contamination and adulteration hazards.

2. List the critical security control points for each of the food product contamination and adulteration hazards, including:
   A. Identification of critical security control points designed to protect product contamination and adulteration hazards in the establishment.
   B. Identification of critical security control points designed to protect product contamination and adulteration hazards, including potential hazards that could occur before, during and after entry.

3. List the critical limits that must be met at each of the critical security control points. Critical limits shall, at a minimum be designed to ensure maximized acceptable reduction of risk as established by the establishment.

4. List the procedures and the frequency with which those procedures will be performed that will be used to monitor each of the critical security control points to ensure compliance with the critical limits.

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5. List the procedures and the frequency with which critical security control points will be tested. This section should remain confidential and should not be made available to other units beyond the security management team.

6. Include all corrective actions that have been developed in response to any determined deficiency identified during planned system testing.

7. Provide for a record keeping system that documents the monitoring of all critical security control points.

8. List the verification procedures for each unit within the establishment and the frequency with which those procedures will be performed.

9. The SARAP plan shall be signed and dated by the responsible establishment individual.

10. The SARAP plan shall be dated and signed:
Upon initial acceptance; Upon any modification ; annually.

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EVOLUTION OF A *SALMONELLA* ENTERITIDIS CONTAMINATION IN TWO LAYER LINES VACCINATED DURING THE REARING PERIOD

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Keywords: *Salmonella* enteritidis, vaccination, laying hens, infection

Abstract

The evolution of the *Salmonella* Enteritidis PT4 infection in laying hens from lines 6 and 15, respectively resistant and susceptible to caecal contamination and vaccinated with « Salenvac », was studied at peak of laying.
Under experimental conditions, caeca and spleens of line 6 were less contaminated than those of line 15 ; the contaminations of livers and ovaries were low, whatever the lines. Vaccination did not eliminate caecal shedding and systemic contamination. Nevertheless, it contributed to reduce the presence of *Salmonella* in the liver, spleen and ovary. Therefore, the possibility of producing contaminated follicles during the laying period is not excluded.

Résumé

L'évolution d'une contamination par *Salmonella* Enteritidis PT4 chez des pondeuses issues des lignées 6 et 15 respectivement résistante et sensible au portage de *Salmonella* Enteritidis et vaccinées avec le vaccin « Salenvac » en période d'élevage à l'âge de 17 et de 21 semaines, a été étudiée au pic de ponte, après une inoculation de $7.2\ 10^8$ *Salmonella* effectuée *per os* à l'âge de 31 semaines.
Dans nos conditions expérimentales, la contamination des caeca et des rates est significativement plus faible chez les pondeuses de la lignée 6 que celle respective de la lignée 15 ; les foies et les ovaires sont très peu contaminés, quelle que soit la lignée. La vaccination n'a pas protégé les pondeuses d'un portage caecal et surtout d'une contamination systémique des autres organes, entraînant une éventuelle possibilité de contamination d'un ou quelques follicules au cours de la ponte.

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Introduction

In order to limit and reduce the number of *Salmonella* Enteritidis toxi-infections, which partly spread through commercial eggs or egg-containing products, various means to fight *Salmonella* Enteritidis infections in flocks have been developped by the shell egg industry. One of them consisted in proposing that the breeders of commercial laying hens should vaccinate hens with an authorized vaccine (O.J. 12/08/1998). As part of the "FAIR6-CT98" program, the incidence of a vaccination with the inactivated vaccine "Salenvac" and its possible interactive action with genetic resistance (against *Salmonella* Enteritidis infections) was studied in two distinct lines of laying hens.

Materials and methods

Animals

Hens from the distinct lines 6 and 15, respectively resistant and susceptible to *Salmonella* contamination (Protais *et al*., 2002), were reared at the "Station de Recherches Avicoles" (INRA). Lines 6 and 15 respectively comprised 32 and 54 *Salmonella* infection-free hens which were randomly allocated to two groups corresponding to the two protected breeding houses (groups 1 and 2), with 2 to 4 hens per cage.

Flock rearing

Until the age of 18 weeks, animals were lit 8 hours per day with a light intensity ranging from 30 to 40 lux. The lighting period was increased by one hour per week until the age of 25 weeks and was kept to 15 hours per day until slaughter.

The layer feed was given *ad libitum* to the animals on arrival. A feed sample was taken from each newly opened feed sack and was placed sterile in a plastic bag with a view to control the *Salmonella* bacteriolgical quality of the feed.

Young hens were vaccinated with the inactivated vaccine "Salenvac" (batch F119102/1) by the intramuscular route in the thigh (0.5 ml) at 17 and 21 weeks of age for the peak of laying occurs later in these lines than in the commercial lines.

Animals were weighed during the study: on arrival before randomization to cages (at 16 weeks of age), at 22 weeks and at inoculation (31 weeks).

The number of eggs laid per cage and the mortality were also recorded.

Inoculation

At the peak of laying, 1 ml of a suspension containing 7.2×10^8 *Salmonella* Enteritidis PT4 5556 P+ per ml was inoculated orally into the crops of the layers in groups 1 and 2 at the age of 31 weeks (D_0).

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Monitoring of environmental contamination

Samples collected prior to inoculation

Before the layers' arrival: in each breeding house, the cages, feeding troughs, walls, filters, trash cans, tables, etc were sampled with wiping cloths and feed and water samples was also collected 113 days prior to inoculation (D_0-113) in order to determine the *Salmonella* status of the breeding houses.

The same controls were carried out approximately 14, 12, 9 and 1 weeks prior to the layers' inoculation in order to determine the flock's hygiene status after vaccinations and prior to inoculation.

Samplings per breeding house after inoculation

Environmental, fecal, feed and water samples were collected 6, 13, 20 and 28 days after inoculation (D_0+6, D_0+13, D_0+20, D_0+28).

Slaughter occured 28 days in average after inoculation. The liver, spleen, caeca and ovary of each laying hen were removed and analyzed.

Search for *Salmonella*

The search for *Salmonella* in the environmental, feed and water samples was performed as follows:

- The preenrichment was carried out in buffered peptone water (BPW) by doing a 1/10 dilution (m/v), except for the water samples which contained a double concentration of BPW. A neutralizer was added to the samples collected after cleaning and disinfection at the rate of 10% the BPW volume. The BPW was incubated at 37°C ± 1°C for 16 to 20 hours.
- The enrichment was performed in a Müller Kauffmann broth incubated at 42°C ± 1°C for 18 to 24 hours. The semisolid Rappaport Vassiliadis medium (MSRV), incubated at 41.5°C ± 1°C for 18 to 24 hours, was added to this medium.
- The selective isolation took place on XLT4 and Rambach agars incubated at 37°C ± 1°C for 18 to 24 hours. The semisolid MSRV medium was again incubated at 41.5°C ± 1°C for an additional 24 hours. Any migration at 24 or 48 hours of incubation on the MSRV medium was isolated on a Rambach agar.
- Finaly, suspect colonies were identified.

The search for *Salmonella* in the organs was carried out as described above, save for the isolation which was done on Rambach agars exclusively.

Salmonella were counted from organ samples diluted in BPW on Rambach agars, save for the caeca for which they were counted on Rambach and XLT4 agars. The count threshold was 2000 c.f.u. per caecum and 100 c.f.u. per liver, spleen and ovary.

Results and discussion

Mortality was observed in line 6 of group 1 and 2 during the young hen rearing period prior to inoculation. After inoculation, no mortality was observed in either group.

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The evolution of the mean body weight of animals from lines 6 and 15 of 2 groups was similar.

The evolution of the laying percentage in each group, both lines taken together, was little influenced by the inoculation, as a number of variations (most probably due to the small number of animals) were observed during the laying period.

The results describing the evolution of the animals' environmental contamination prior to and after inoculation and the evolution of the organ contamination regarding the animals' genetic origin are shown in Tables 1 and 2.

Prior to inoculation:

No contamination of the environment or animals was observed, neither prior to the pullets' arrival, nor before or after the two vaccinal injections, nor prior to inoculation of layers. Therefore, observations did result from the experimental inoculation.

After inoculation:

The presence of *Salmonella* Enteritidis PT4 was revealed in various environmental samples in groups 1 and 2, which clearly shows that vaccination cannot totally prevent the risk of bacteria shedding, particularly when a high dose of *Salmonella* has been inoculated or when the animals are susceptible.

The rate of *Salmonella* Enteritidis isolation in the organs for each line in the breeding houses is indicated in Table 2. Contamination rates of liver and ovary were very low, as observed by Protais *et al*. (2002), which may account for the absence of any significant discrepancy between the lines.

The caeca were more often contaminated and line 15 as a whole tended to be more contaminated. This discrepancy could be observed in all groups; indeed, after verifying the homogeneity of groups 1 and 2 using a Zelen test, the calculation of the common odd-ratio revealed significant discrepancies in caeca contamination between lines 6 and 15.

The level of contamination was rather low : *Salmonella* Enteritidis PT4 was counted in only two caeca. Both came from animals from line 15 in group 2. The mean value of this count was 4.03 log c.f.u. per caecum, which represents 3.07 log c.f.u. when brought to a gram of organ.

However, line 6 showed a milder contamination of spleens by *Salmonella* Enteritidis PT4 which was significant in group 1, at the limit of the 5% threshold in group 2 and highly significant in both groups taken together ($P<0.001$). *Salmonella* Enteritidis PT4 was identified, on the one hand, in one or several organs of respectively 7.7% and 51.9% of layers from lines 6 and 15 of group 1 ($P<0.013$) and on the other hand, in respectively 29.4% and 74.1% of layers from lines 6 and 15 in group 2 ($P<0.005$). These results confirm those from a former experiment which showed that lines 6 and 15 were respectively resistant and susceptible to *Salmonella* Enteritidis caecal contamination.

In our experimental conditions, vaccination of the layers did not fully protect animals with a *Salmonella* contamination in the caeca, and even less when the contamination

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was located in the liver, spleen and ovary. This systemic contamination suggests a risk of potential contamination of the follicles during the laying period, although this risk should be relatively lower than in non-vaccinated animals (Protais *et al*., 2002 ; Feberwee *et al*., 2001, 2002).
In our experimental conditions, laying hens from lines 6 (resistant) and 15 (susceptible), vaccinated with the inactivated vaccine "Salenvac" by the intramuscular route at 17 and 21 weeks of age and subsequently contaminated orally with $7.2x10^8$ *Salmonella* Enteritidis PT4 at 31 weeks of age, shed *Salmonella* in the environment.
Four weeks after inoculation, the contamination rates of livers and spleens were very low and similar. Besides, caeca and spleens of layers from line 6 were significantly less contaminated by *Salmonella* Enteritidis PT4 than those from line 15, which confirms that lines 6 and 15 show discrepancies in their susceptibility to *Salmonella* Enteritidis PT4 contamination.

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Table 1 : Evolution of environmental contamination during the experiment

	D_0+6		D_0+13		D_0+20		D_0+28	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Cages (4)[a]	4[b]	4	4	1	1	0	1	0
Feeding troughs (4)	4	3	1	0	0	0	0	0
Droppings (4)	4	4	4	4	2	2	3	1
Feed (4)	4	4	2	0	2	0	2	2
Water (2)	2	2	1	0	0	0	1	0
Environment (4) (Filters, walls…)	4	4	3	2	1	1	1	0

(4)[a] : 4 samples collected
4[b] : 4 positive samples

Table 2 : Organ contamination rate in each group

	Group 1		Group 2		χ_2	
	Line 6	Line 15	Line 6	Line 15	Group 1	Group 2
Caeca	1/13[a] (7.7 %)	9/27 (33.3 %)	4/17 (23.5 %)	12/27 (44.4 %)	3.08 NS (0.12)[b]	1.97 NS (0.21)
Liver	0/13	0/27	0/17	2/27 (7.4 %)	-	1.32 NS (0.52)
Spleen	0/13	9/27 (33.3 %)	1/17 (5.9 %)	9/27 (33.3 %)	5.59 * (0.04)	4.48 NS (0.06)
Ovary	0/13	0/27	1/17 (5.9 %)	0/27	-	1.63 NS (0.39)
1 organ +	1/13 (7.7 %)	14/27 (51.9 %)	5/17 (29.4 %)	20/27 (74.1 %)	7.30 ** (0.013)	8.48 ** (0.005)

a: 1 positive caecum / 13 caeca analyzed
(7.7 %) : percentage of positive samples
b: ( ): P ; NS : not significant ; * $P < 0.05$; ** $P < 0.01$

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USE OF PAIRED CARCASS HALVES IN TESTING OF ANTIBACTERIAL TREATMENTS

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Keywords: antibacterial treatments, carcass halves, experimental design

Abstract

Using counts of chicken carcass bacteria from a previous experiment, 100 data sets were generated to test the efficiency of a completely randomized design (using counts from whole carcasses) versus a random block design (using paired left and right carcass halves) for testing effects of treatments on numbers of bacteria. Mean reductions in counts to obtain a significant difference between control and treatment groups for aerobic bacteria, coliforms, and *E. coli* were 0.44, 0.51, and 0.63 for the randomized design and 0.25, 0.26, and 0.35 for the random block design, respectively, with significant differences between the two designs for all three types of bacteria.

Résumé

En utilisant les dénombrements de bactéries de carcasse de poulets issus d'une autre expérience, 100 séries de données ont été générées pour tester l'efficacité d'une approche complètement aléatoire (utilisant les populations de bactérie provenant de carcasses entières) en comparaison à une approche aléatoire en séries opposées (utilisant un arrangement deux par deux de moitiés de carcasses gauche et droite) afin de tester les effets de traitements sur un grand nombre de bactéries. Les réductions moyennes en population nécessaires pour obtenir une différence significative entre les groupes de contrôle et les groupes "traités" de bactéries aérobies, coliformes, et E. coli étaient 0.44, 0.51, et 0.63 pour l'approche aléatoire, et 0.25, 0.26, et 0.35 pour l'approche aléatoire en séries opposées, avec des différences significatives entre les deux approches pour les trois genres de bactéries.

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Introduction

To reduce the numbers of pathogenic bacteria in food, many different treatments have been tried to kill or prevent growth of bacteria that may be present. Antibacterial treatments that have been applied to chicken carcasses and chicken meat have included high and low temperatures, hot water, steam, high pressure, herbal formulations, essential oils, beneficial bacteria, irradiation, and chemicals such as organic acids, chlorine, chlorine dioxide, trisodium phosphate, and others.

Experiments to test the efficacy of antibacterial treatments have usually compared numbers of bacteria on carcasses that were randomly assigned to treatment or control groups, although there is wide variation in numbers of bacteria found on chicken carcasses. Two studies have reported a high correlation between numbers of bacteria on left and right halves of the same carcass (Izat et al, 1990; Cason and Berrang, 2002), however, so treating half carcasses and comparing results between paired halves should be a more sensitive test. The present experiment used raw data from a previous study to generate random control and treatment groups to compare a completely random design using whole carcasses against a random block design using paired half carcasses.

Material and Methods

Data from the study of Cason and Berrang (2002) were available for use. The data consisted of counts (log10 cfu/ml) of aerobic bacteria, coliforms, and *Escherichia coli* in rinses of left and right halves of 45 carcasses taken from a commercial processing plant before chilling. The carcasses had been split aseptically and then rinsed in 400 ml of sterile water. Experimental data sets for the random design were generated by randomly assigning counts of 10 whole carcasses to a control group and 10 to a treatment group. For the random block design, 10 carcasses were randomly selected and the right and left halves of each carcass were randomly assigned to control or treatment groups. Both the random and random block designs would have involved determination of bacterial counts in 20 different samples, so the amount of work in the laboratory would have been the same for each design. The process was repeated until 50 different data sets had been generated for each design, with counts for 3 types of bacteria.

Analysis of variance was conducted for each type of bacteria in each of the 100 data sets. If there was no statistical difference ($P < 0.05$) between the treatment and control groups, 0.1 was subtracted from all counts in the treatment group and the process was repeated until a statistical difference between control and treatment

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groups was found. The number of 0.1 reductions necessary to reach statistical significance was recorded, and the numbers from the whole and half carcass data were tested by analysis of variance in a completely randomized design. All randomizations and statistical analyses were done using the SAS statistical program (SAS Institute, 1987).

Results and discussion

Table 1 shows the mean counts for aerobic bacteria, coliforms, and *E. coli* in the original 45 carcasses and in the generated data sets. Means for the generated data sets indicated that the randomization process was unbiased. For the 150 comparisons that were performed between the two designs (50 different data sets for each and 3 types of bacteria), 8 tests were significantly different without any subtractions from the counts in the original random sorting. That result (8/150 = 0.053%) compares well with the alpha level of 0.05 that was chosen as the measure of significance.

Table 2 shows the mean reduction in log counts of the treatment groups necessary to obtain a significant difference from the control groups. For all three kinds of bacteria, the mean reduction in counts to obtain a difference was significantly less for the random block design using paired half carcasses than for the random design using whole carcasses.

Random block designs are known to be more efficient than completely randomized designs, but the blocking factor must have a real influence on the variable being tested. This experiment demonstrates that the relationship between counts of bacteria on opposite sides of the same carcass is strong enough to justify use of carcass halves in a random block design. Compared to completely randomized designs, tests based on random block designs will be more sensitive to treatment effects with the same number of observations or as sensitive with fewer observations.

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Table 1. Means counts (log 10 cfu/ml of rinse) of bacteria in the original data and in randomly selected data sets used for testing random designs with whole carcasses versus random block designs with paired half carcasses

Bacteria	Original data	Randomly selected data	
		Control	Treatment
Whole carcasses			
Aerobic	3.04	3.01	3.05
Coliforms	1.89	1.88	1.88
E. coli	1.36	1.35	1.36
Half carcasses			
Aerobic	2.71	2.72	2.73
Coliforms	1.57	1.58	1.58
E. coli	1.01	1.03	1.03

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Table 2. Mean reduction in counts (log 10 cfu/ml of rinse) of bacteria in treatment groups necessary to obtain a significant difference ($P < 0.05$) from control groups

Design	Aerobic	Coliforms	*E. coli*
Randomized (whole carcasses)	0.44a	0.51a	0.63a
Random block (half carcasses)	0.25b	0.26b	0.35b

a,b For each type of bacteria, means labeled with different letters are significantly different ($P < 0.001$).

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MICROBIAL ECOLOGY OF COMMERCIAL BROILER DEBONING OPERATIONS AND POTENTIAL IMPACT ON FOOD SAFETY

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Keywords: *Campylobacter jejuni*, microbial ecology, deboning operations

Abstract

Research was conducted to define the microbial ecology of poultry products and equipment surfaces in commercial broiler deboning operations, and to determine effects of processing on predominant bacterial types. Sampling of whole carcasses, breast meat, and equipment surfaces yielded 600 isolates. Among these isolates, 34 different genera representing 98 different species were found. *Staphylococcus, Pseudomonas, Flavobacterium* and *Acinetobacter* were the predominant genera; however, the overall bacterial community varies by sample type. Clear patterns of cross contamination were observed. Isolates were assayed to determine their ability to inhibit growth of *Campylobacter jejuni*, and 62 were found to be antagonistic to *C. jejuni.* Isolates of *Pseudomonas aeruginosa* were highly inhibitory to growth of the pathogen. Microbial ecology and changes that occur with commercial deboning procedures may impact the incidence of *C. jejuni* on processed poultry products.

Résumé

Ces travaux ont été conduits afin d'établir l'écologie microbienne des produits de volailles et des surfaces de travail dans une usine de désossement de volailles et pour déterminer l'effet du processus sur la flore dominante. L'échantillonnage a porté sur des carcasses, des filets et des surfaces de travail, et a permis la récolte de 600 isolats bactériens. 34 genres représentant 98 espèces bactériennes ont été identifiés. *Staphylococcus, Pseudomonas, Flavobacterium* et *Acinetobacter* sont les genres dominants, mais la composition bactérienne varie par type d'échantillons. Des profils de contamination croisés ont été clairement mis en évidence. Des isolats ont été testés pour leur capacité à inhiber la croissance de *Campylobacter jejuni* et 62 d'entre eux ont montré cet antagonisme. Les isolats de *Pseudomonas aeruginosa* sont fortement inhibiteurs de *Campyloacter.*

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L'écologie microbienne qui s'installe au cours du désossement peut influencer comportement de *c.jejuni* dans les viandes de volailles.

Introduction

Poultry processing methods continue to evolve to meet consumer demand for a wide variety of convenient ready-to-cook products. While there are abundant data on factors affecting bacterial contamination of poultry during initial slaughter and processing, there are little data on contamination during further processing steps such as cut-up and deboning. Moreover, poultry is a known reservoir for the pathogen *C. jejuni*, and there is a high prevalence of this pathogen on whole broiler carcasses, while recent reports indicate a significantly lower prevalence of *C. jejuni* on further processed poultry (Davis *et al*., 2002). The reason for this difference is unknown, however competition by other bacteria may, in part, account for the observed lower prevalence. Thus, research is needed to further define the microbial ecology of poultry products in the modern market place, and to determine the potential of this ecology to influence overall safety of these poultry products.

Material and methods

In experiment 1, samples were taken at random at the postchill-debone lines of the same commercial processing facility over three visits. Collection at each sampling time consisted of 20 whole carcasses (W), 20 breast skins (SK), 20 breast meats (BM), and 20 equipment (E) samples. Whole carcasses were collected immediately after immersion chilling. Skin samples were collected at the skin transfer belt. Breast samples were gathered immediately after their removal from carcasses. Equipment surfaces were sampled by swabbing, and included debone belt, splash plate behind debone belt, splash plate to the side of debone line, overhead drip pan, knife sharpener, drip pan under cone line, skin transfer belt, employee apron, employee glove, and cone line. Samples (W, SK, BM) were individually placed in sterile plastic bags, with 400 ml buffered peptone water (BPW), and shaken for one minute. Equipment surfaces (E) were swabbed using templates (160 cm^2). Sterilized gauzes moistened with buffered peptone water were used to swab the areas inside the templates. After swabbing, gauzes were placed in 400 ml BPW. Each rinse fluid was serially diluted using Butterfield's phosphate diluent and spiral plated (50µ) onto tryptose soy agar (TSA). Plates were incubated at 37C for 24 h. Isolated colonies exhibiting different morphology were selected, and streaked for isolation on fresh TSA. The total number isolates obtained from the various sample types are as follows:

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Table 1. Number of isolates from different sampling location.

Whole carcass samples (C)	210
Breast skin samples (SK)	112
Breast meat (BM)	244
Equipment surface samples (E)	104
Total	670

Isolates were identified based on fatty acid composition using the Sherlock™ system (MIDI, Inc., Newark, DE, USA). Isolates were streaked onto TSA with 5% sheep blood (Remel) and incubated at 35C for 24 h. After incubation, there were five steps in the preparation of GC-ready extracts from cell cultures for fatty acid composition analysis (MIDI, Inc.), namely harvesting, saponification, methylation, extraction, and base wash. Harvested cells were removed from culture medium. One loopful (approximately 40 mg) of live wet cells harvested from the most dilute quadrant exhibiting confluent growth (late log phase) along the streaking exit was used for identification procedure. Harvested cells were placed into a clean dry 13 mm N 100 mm screw cap culture tube. The next step was saponification, in which cells were lyzed to liberate fatty acids from cellular lipids which were subsequently methylated to convert fatty acids to fatty acid methyl esters to increase the volatility of the fatty acids for GC analysis. Following methylation, fatty acid methyl esters were removed from the acidic aqueous phase and transferred to an organic phase. A mild base solution was then added to the sample preparation tubes to remove free fatty acids and residual reagents from the organic extract prior to chromatographic analysis. Fatty acids extracted from the isolates were automatically quantified and identified by the Sherlock™ software to determine the fatty acid composition. The fatty acid profile was then compared to a library of reference organisms to determine the identity of the unknown isolate. Isolates were identified to the species level.

In experiment 2, the amount of inhibition that other poultry bacterial isolates have on *C. jejuni* was determined. In this experiment, two assays were used: agar and broth procedures. For the agar assay, 600 bacterial isolates collected from a commercial deboning line (from experiment 1) were used. TSA (30ml) was allowed to solidify in an agar plate. After solidification, five wells were punched into the agar. Three of these wells received 50µl of TSA containing 10^8cfu/ml of the isolate being tested for inhibition of *Campylobacter*. One well received 45µl of TSA plus 5µl of acetic acid (3N). The final well received 50µl of TSA. These plated were incubated at 35C for 24h. After incubation, the plates were subjected to UV light for 2h. After this exposure, the plates were overlayed with 10 ml of Campy-cefex agar containing 10^7cfu/ml of *C. jejuni*. The plates were then incubated microaerophilically at 42 C for 48h. After incubation, the plates were examined for zones of inhibition. 62 isolates representing 26 species produced zones of inhibition and were subjected to the broth assay. For the broth assay, each target isolate was inoculated (10^9 cfu/ml) into 9 ml Brucella broth and incubated microaerophilcally at 42C for 24h. These isolates were

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then plated onto both TSA and Campy-Line media. These plates were incubated at 42C for 48h. This was to ensure that the target organism did not grow on the Campy-Line agar. To determine the amount of inhibition of each isolate, 1ml of each isolate (10^9 cfu/ml) and 1 ml of *C. jejuni* (10^8 cfu/ml) were inoculated into 8 ml Brucella Broth. This broth was incubated at 42C for 24h. After incubation, the mixture was plated onto Campy-Line agar and incubated at 42C for 48h. For control, 1ml of *C. jejuni* was inoculated into 9ml Brucella Broth and incubated at 42C for 24h. This broth was then plated onto Campy-Line agar and incubated at 42C for 48h. To measure inhibition, the population of the mixture of *C. jejuni* and the target organism was subtracted from the population of pure *C. jejuni*.

Results and Discussion

In experiment 1, a total of 670 isolates (210 W, 112 SK, 244 BM, 104E) were obtained based on different morphology on TSA. Of these, 600 isolates (193 W, 87 SK, 222 BM, 98 E) were identifiable using the Sherlock™ system. Among the 600 isolates identified, there were 34 different genera, representing 98 different species. *Staphylococcus, Pseudomonas, Flavobacterium*, and *Acinetobacter* were the most predominant genera (Table 2). Processing environment affected bacterial population as evidenced by observed increases in the proportion of psychrotrophic bacteria during sequential broiler deboning operations (Table 3). Based on this shift in bacterial types it appears that equipment surfaces serve as the primary source of psychrotrophic bacteria on breast skin and meat. The high prevalence of staphylococci on whole carcasses (42.49%) and breast skin (19.54) and the low prevalence of these bacteria on equipment surfaces (10.20%) and breast meat (7.66%) indicate that the plant environment was not the main source of the staphylococci contamination on broiler products. Enterobacteriacea were found from all the four types of samples, however, cold tolerant genera of the family Enterobacteriacea such as *Serratia, Enterobacter, Citrobacter* were found at high proportion on equipment surfaces, and thus also found at high proportion of breast meat. The large number of genera found in breast meat (data not shown) indicated that significant cross contamination occurs during the deboning operation. Thus, steps to minimize this cross contamination would likely lead to product of improved microbiological quality.

In experiment 2, sixty-two of the initial 600 isolates produced zones of inhibition in the agar assay of the experiment (Table 4). These 62 isolates represented 26 different species of bacteria. In the broth assay, the amount of inhibition was measured. Of the 62 isolates, 26 (representing 15 species) reduced the growth of *C. jejuni* by 1-2 $\log_{10}$ cfu/ml. Twenty six isolates (representing 16 species) reduced the growth of *C. jejuni* by 2-3 $\log_{10}$ cfu/ml and 10 isolates (representing 1 species) reduced the growth of *C.*

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jejuni by > 3 $\log_{10}$ cfu/ml. Chemical substances released by these microorganisms may account for the inhibitory effects on *C. jejuni*, including antibiotics, bacteriocins, hydrogen peroxide, and some organic acids. Nonetheless, presence of other poultry-borne bacteria influence the survival of *C. jejuni*, which may affect the ability of this pathogen to persist on raw poultry meat.

Table 2. Distribution of genera among 600 bacterial isolates from whole carcasses, breast skins, breast meats, and equipment samples.

Genera	%	Genera	%
Staphylococcus	21.00	*Corynebacterium*	0.50
Pseudomonas	17.67	*Gordona*	0.50
Flavobacterium	15.83	*Legionella*	0.50
Acinetobacter	13.00	*Nocardia*	0.50
Bacillus	5.00	*Proteus*	0.50
Escherichia	4.67	*Sphingobacterium*	0.50
Enterobacter	3.50	*Hafnia*	0.50
Micrococcus	3.33	*Rothia*	0.33
Klebsiella	2.33	*Acidovorax*	0.33
Serratia	2.17	*Alcaligenes*	0.17
Citrobacter	1.33	*Bergeyella*	0.17
Leclercia	1.33	*Haemophilus*	0.17
Neisseria	1.00	*Moxaxella*	0.17
Salmonella	0.83	*Morganella*	0.17
Aeromonas	0.83	*Providencia*	0.17
Enterococcus	0.50	*Psychrobacter*	0.17
Shigella	0.50	*Vibrio*	0.17

Table 3. Distribution of predominant genera from different sampling locations.

Genera	Whole carcass (%)	Equipment surface (%)	Breast skin (%)	Breast meat (%)
Pseudomonas	10.88	24.48	22.99	18.47
Flavobacterium	9.32	14.29	13.79	22.97
Acinetobacter	4.15	31.06	11.49	13.06
Staphylococcus	42.49	10.02	19.54	7.66
Escherichia	7.25	2.04	4.60	3.06
Enterobacter	3.63	3.06	1.15	4.50
Serratia	0.52	3.06	1.15	3.60
Citrobacter	1.04	1.02	2.30	1.35
Neisseria	0.00	3.06	0.00	1.35

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Table 4. Inhibition of *C. jejuni* using agar bioassay.

Genera	Species	No of isolates producing visible inhibition	Zone size (mm)	Growth reduction (log_{10} cfu/ml)
Pseudomonas	*P. aeruginosa*	12	6.0-9.5	2.30-5.77
	P. putida	7	1.5-5.0	1.04-2.66
	P. maltophilia	3	1.0-1.5	1.01-1.67
Flavobacterium	*F. gleum*	5	6.0-9.5	1.20-2.04
Acinetobacter	*A. johnsonii*	3	1.0	1.95-2.40
	A. lwoffii	1	2.5	2.39
Staphylococcus	*S. aureus*	1	1.5	2.08
	S. cohnii	5	3.0-5.0	1.60-2.32
	S. saprophyticus	1	5.0	2.51
	S. scuiri	3	1.0-1.5	1.23-1.85
	S. xylosus	3	1.0-1.5	1.24-2.32
Micrococcus	*M. varians*	2	1.5	2.35-2.36
Enterococcus	*E. casseliflavus*	1	1.0	1.52
Citrobacter	*C. braakii*	1	9.5	1.57
	C. freudii	1	4.5	2.43
Escherichia	*E. coli*	2	3.5-10.0	1.30-1.89
Enterobacter	*E. cloacae*	1	1.0	1.09
Hafnia	*H. alvei*	1	1.5	1.86
Klebsiella	*K. oxytoca*	1	1	1.04
	K. pneumoniae	2	1.5	1.05-1.45
Vibrio	*V. fluvialis*	1	1	2.28
Bacillus	*B. circulans*	1	2	2.20
	B. licheniformis	1	7.5	2.94
Moraxella	*M. osloensis*	1	3.5	2.80
Neisseria	*N. sicca*	1	9.5	2.41
Nocardia	*N. brasiliensis*	1	1.0	1.44

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HOW MUCH HAS THE TABLE EGG CHANGED OVER THE LAST 40 YEARS ?

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Keywords: Eggs, Layer Strain, Egg Age, Hen Age

Abstract

The eggs produced from different closed, random-bred strains of commerical laying hens which were reared and managed under the same environmental conditions were compared to see how they would withstand a surface challenge of *Salmonella* Enteritidis and *Pseudomonas fluorescens*. The results of the study show that genetic selection has altered the egg's ability to withstand microbial contamination and penetration during storage. Additionally, the results indicate that genetic selection has produced larger eggs containing a lower percentage of yolk while overall egg quality has been maintained or improved. With today's food safety concerns, it may be beneficial for breeders to examine microbial intergrity of shell eggs as a screening tool to enhance table egg safety and quality.

Résumé

Des œufs issus de différentes lignées génétiques commerciales élevées dans des conditions standardisées ont été comparés pour leur capacité à empêcher la pénétration de Salmonella Enteritidis et de Pseudomonas fluorescents à travers la coquille. Les résultats de cette étude montrent que la sélection génétique a altéré la résistance de la coquille à la pénétration des bactéries. Parallèlement, il a été montré que la sélection a permis de produire des œufs plus gros contenant un % moindre de jaune, alors que la qualité générale des œufs était dans le même temps augmentée ou maintenue. Avec les problèmes de santé publique actuels, les sélectionneurs devraient se préoccuper de cribler leurs nouvelles souches sur la base de la résistance de la coquille à la pénétration bactérienne.

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Introduction

Eggs have changed in a number of ways over the years. They have become larger and rounder in shape. Numerous production and processing changes have occurred within the commerical egg industry over the past 40 years that have affected the quality and safety of eggs being delivered to consumers in the United States (Bell, 1998). However, the recognition of *Salmonella* Enteritidis (SE) as an emerging pathogent in eggs (St. Louis et al., 1988 ; Gast and Beard, 1990) led to perhaps the most dramatic shift in the consumer definition of egg quality in recent years (Thornton, 1991). Prior to SE, consumers defined egg quality in physical and visual terms (i.e. size of the air cell, color or the yolk, height of the albumen) and few consumers expressed concern about the microbial load contained on or within commercially processed eggs. Confirmation of Grade A shell eggs as a major source of SE infections (Gast, 1994 ; Humphrey, 1994) led to a shift in consumer attention and research efforts.

Much of the work conducted in the past has encompassed egg safety and cleanliness which can be diffcult to compare due to differences in rearing and management practices of the laying hens. The current study was designed to compare the eggs produced from different closed, random-bred strains of commericial laying hens which were reared and managed under the same environmental conditions.

Material and Methods

Eggs were collected from three Ottawa Control Strains, provided by Agriculture Canada, and a current commercial laying strain. Each strain was hatched and reared under identical environmental and management conditions (Anderson, 1996). The random-bred control strains utilized were strins 5 (CS5), 7 (CS7), and 10 (CS10) as described by Gowe et al. (1993) and Fairfull et al. (1983). Selection was closed in these strains in 1950, 1959, and 1972, respectively. The current commercial stock (CCS) was a 1993 laying stock with a common ancestral linkage to the control strains. Molt was induced in all strains at 62 weeks of age.

Eggs were collected monthly over a 62 week laying period and analyzed for egg, albumen, shell and yolk weight ; albumen proteins, solids and pH ; percent yolk solids and fat ; Haugh units ; and specific gravity.

Egg samples were collected for microbial analysis from all strains when hens were 32, 45, 58, 71 and 84 weeks of age. *Pseudomonas fluorescens* (PF) and nalidixic acid resistant SE (Brant et al., 1965) were used to inoculate eggs. Eggs from each of the four hen strains were inoculated with SE, PF, or a combination of SE and PF. After

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stoarge at room temperature, contamination levels were determined for the exterior surface, air cell, egg contents and within the shell.

Results and discussion

Although the eggs increased in size, no significant differences were found between strains for specific gravity or percent shell weight. Haugh units were significantly higher in the CS10 and CCS strains than in the other strains. No significant differences were found in albumen solids, protein, pH or yolk solids. The results indicate that genetic selection has produced larger eggs containing a lower percentage of yolk while overall egg quality was maintained or improved.

SE and PF survived at different rates on the shell surface with as much as a 1 log difference during a given collection period. Egg content counts tended to be higher than egg shell counts in PF, whereas the opposite was true for SE. These data suggest that PF is a primary invader of eggs that is more capable of contaminating egg contents through the shell membranes than SE. The PF and SE data suggest that bacterial contamination of air cells, shells, and egg contents is more easily achieved in eggs from older hens than from younger hens. There were also differences between strains.

Interior, egg contents and shell contamination levels of both SE and PF increased during storage. There were no apparent increases in the infectivity of SE or PF in the presences of the other organism. PF was a poor survivor on the shell surface under storage conditions. Through the five week storage, the CS10 eggs were better able to maintain their microbial integrity after inoculation. Eggs from CS5 and CCS were the most easily contamianted of the strains compared. Genetic selection appears to have altered the ability of a hen to produce an egg with strong microbial defenses.

The overall results of this study suggest that genetic selection has altered the ability of eggs to resist microbial contaiination and that screening for microbial integrity shold be considered in the selection process among the laying egg breeders.

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A SURVEY ON INSTITUTIONAL USERS OF SHELL EGGS AND EGG PRODUCTS IN FLANDERS

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Keywords: institution, questionnaire, *Salmonella*, shell eggs, egg products

Abstract

A survey assessed the handling of shell eggs and processed eggs from purchase until serving to potentially vulnerable people in institutions. Shell eggs and egg products are used in respectively 94% and 52% of the institutions. The questionnaire listed a series of egg dishes, which were classified afterwards in three risk categories. Of the high-risk dishes, shell eggs are particularly used for addition to mashed potatoes, for preparing soft-boiled eggs and eggs sunny-side-up and for inclusion in hot sauces. Egg products are purchased on a rational base. The distribution of storage temperature, storage time and preparation frequency of high-risk meals were combined using a Monte Carlo resampling technique, which indicated that the majority of the samples (98.5%) fell into a low-hazard category.

Résumé

Une enquête sur les conditions de manipulation des œufs en coquille et des ovo produits entre l'achat et le service a été menée auprès d'institutions d'hébergement des personnes à risque. Les œufs en coquilles et les ovo produits sont utilisés respectivement dans 54 et 52 % des institutions. Le questionnaire comprenait une liste de plats à base d'œufs qui ont été classés par la suite en 3 niveaux de risques. Dans la catégorie des plats à haut risque, les œufs en coquilles sont utilisés soit pour enrichir les purées, soit sous forme d'œufs à la coque ou sur le plat, soit en adition dans les sources chaudes. Les ovo produits sont utilisés sur une base plus rationnelle. La distribution des températures de stockage, des temps de stockage et des fréquences de

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préparations à hauts risques ont été croisés par la technique de Monte Carlo. 98.5% des échantillons entrent dans la catégorie à faibles risques.

Introduction

When associated with food borne outbreaks, *Salmonella* infection usually leads to gastroenteritis. The infective dose may be as high as 10^7-10^9 organisms or < 100 organisms. The attack rate ranged from 16-50% (Kothary and Babu, 2001). The symptoms of illness are usually mild in healthy adults, but can be more severe among small children and the elderly or infirm (Cox *et al.* 2000). The elderly experience a mortality ratio from *Salmonella* infections of 3.8% (Haas et al., 1999). Of the food borne disease outbreaks in nursing homes, *Salmonella* was the most frequently reported pathogen. *S. enteritidis* outbreaks accounted for 56% of the *Salmonella*-associated deaths. The implicated food vehicles in *S. enteritidis* outbreaks were made with eggs or prepared with equipment contaminated with eggs (Levine et al., 1991). Since standard cooking practices may not eliminate high levels of *S. enteritidis*, storage temperatures may be very important in controlling *Salmonella* infections and maintaining egg quality (Schoeni et al., 1995). The use of processed eggs can solve many problems regarding preservation and microbiological safety (Cotterill and McBee, 1995). In recent years, new convenience egg products designed to meet the needs of the home and institutional markets have been introduced (Baker and Bruce, 1994).

Material and Methods

Eggs. A questionnaire with 35 questions was distributed among 125 institutions. Four main categories of questions can be distinguished: socio-economical information, purchase of eggs or egg products, storage and preparation of eggs or egg products, and production and distribution.

Results and Discussion

Socio-Economical Information. The overall response rate for the questionnaire was 54% with 68 questionnaires being returned; 21 from care homes, 20 from acute hospitals, 14 from handicapped institutions, and 10 from crèches.

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The Purchase. In 45% of the institutions both shell eggs and egg products are bought and in 49% only shell eggs are bought. Egg products only are purchased in few institutions (ca. 6%). Differences exist in the purchasing behavior depending on the type of institution. A cooled van is mostly used for the delivery of shell eggs and/or egg products. Mostly (73%), the eggs and/or egg products are never controlled microbiologically. When problems arise, the microbiological quality is controlled in 23% of the institutions. A witness dish is present in 62% of the institutions.

The Storage. In most institutions, the *eggs* are stored in the refrigerator or cool storage room (75%) (2-7°C, mean 4.1°C). In 15% of the institutions, the eggs are stored in a cold place (8-17°C, mean 11.4°C) and at room temperature (16-20°C, mean 18.0°C) in 10% of the institutions. The average keeping time of the eggs is 1 to 2 weeks (48%). In 34% and 8% of the institutions, the eggs are kept for 7 days and 2 to 3 weeks, respectively. A χ^2 test showed that there is no significant relationship between the storage time and the storage temperature. The *egg products* are mostly stored in the refrigerator or cool storage room (97%) having an average temperature of 3.9 ± 0.5°C. The egg products are mostly kept for 7 days (39%), 1 to 2 weeks (23%) and until the end of shelf life (26%).

The Preparation. The nineteen dishes made from *shell eggs* that were mentioned in the survey were grouped into three categories: high-risk, medium-risk and low-risk egg dishes (Table 1). This categorization was based on the temperature-time trajectory that the eggs go through. It was shown that when egg products are additionally bought, high-risk and medium-risk dishes are less prepared than when only shell eggs are bought: 11% and 16% more institutions do never prepare one of the high-risk and medium-risk dishes, respectively. For low-risk dishes, there appears to be no effect. The institutions preparing the dishes from either shell eggs or egg products and those preparing these from shell eggs are summarised in Table 1. Following the advice issued by the Flemish Government, it should be avoided to give vulnerable groups of people soft and medium-boiled eggs, raw egg containing meals, and lightly cooked meals (Yde et al., 1997).

The Production And Distribution. In 91% of the institutions, a coupled production system is used. The delay time between portioning and consumption is less than 15 min in 34% of the institutions and between 16 and 30 min in 31% of the institutions.

Quantifying The Risk Level Of Consumer Egg Handling. By combining the histograms of temperature, storage time and high-risk egg dishes, a distribution of consumer risk behavior with respect to egg handling was obtained as described by Grijspeerdt et al. (1999).

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Table 1. Categorization of egg dishes and percentage of institutions preparing these dishes from shell eggs or egg products

	Shell eggs	Egg products	
	Institutions preparing dish from shell eggs (%)	Institutions preparing dish from egg products (%)	Frequency dish is made from egg products
High-risk dishes			
Mashed potatoes	23	17	0.42
Soft-boiled eggs	17	n.r.[1]	n.r.[1]
Hot sauces	15	9	0.37
Eggs sunny-side up	14	n.r.[1]	n.r.[1]
Cold sauces	11	9	0.46
Cold desserts	11	6	0.36
Poached eggs	3	n.r.[1]	n.r.[1]
Soup	2	0	0
Medium-risk dishes			
Scrambled eggs	52	8	0.13
Omelet	48	14	0.22
Hot desserts	9	5	0.33
Medium-boiled eggs	8	n.r.[1]	n.r.[1]
Soufflé	2	0	0
Low-risk dishes			
Hard-boiled eggs	65	n.r.[1]	n.r.[1]
Garniture	62	22	0.23
Pastry	40	5	0.10
Fish salads	32	12	0.28
Meat ball/loaf	28	2	0.05
Meat salads	28	6	0.18
Eggs for deep frying pan dishes	19	n.r.[1]	n.r.[1]
Meat pie	2	2	0.50

[1] n.r., not relevant

The monthly number of preparations for all dishes prepared by each institution was summed. This gave values between 0 and 31. All histograms were rescaled between 0 and 1, with 0 being the lowest value given (4°C, 5 days of storage, 0 for dishes) and 1 being the highest value given (20°C, 24.5 days of storage, 31 for dishes).

Since no significant interactions were found between the temperature-time-dish distributions, the use of uncorrelated resampling (multiplying temperature-time-dish

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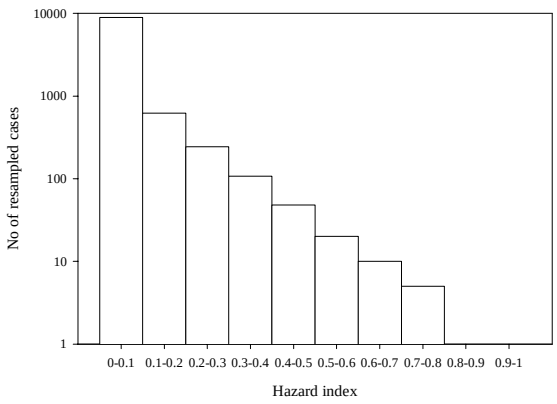

Fig. 1. The hazard index histogram

rescaled values) was allowed. This was done with a Monte-Carlo simulation algorithm using a Latin Hypercube sampling scheme in 10,000 iterations. The resulting distribution can be considered as some kind of "hazard index". The histogram is shown in Fig. 1. The majority (98.5%) of cases falls in the low-hazard (0-0.33) category. Only 1.4% of cases belong to the medium-hazard (0.33-0.66) category and 0.1% of cases belong to the high-hazard (0.66-1) category. It should be emphasized that these simulation results should not be considered as absolute values, but must be considered as an illustration that can provide useful insight.

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APPLICATION OF LOW-RESOLUTION ^{1}H NMR SPECTROSCOPY (LR ^{1}H NMR) FOR STUDYING EGG DEFENCE AGAINST MICROBIAL GROWTH

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Keywords: LR ^{1}HNMR spectroscopy, shell eggs, Haugh units, microbial growth

Abstract

LR ^{1}H NMR was used to investigate microbial growth in eggs in relation to egg age and hen age. Standardized eggs from caged 'Lohmann Brown' hens were inoculated with a defined pool of bacteria and stored under defined conditions. Characteristic changes in the NMR relaxation times indicated bacterial growth to levels > 10^7 - 10^8 c.f.u./g of egg content. These changes frequently correlated with optical-olfactorial deviations of the egg contents. The resistance of the eggs against microbial growth decreased with increasing storage time before inoculation. Eggs from younger hens showed a higher resistance than the eggs from older hens. The percentage of 'NMR-positive eggs' increased with decreasing Haugh Units.

Résumé

La résonance magnétique nucléaire à basse résolution, LR ^{1}H NMR a été évaluée comme technique de mesure de la croissance microbienne dans l'œuf en fonction de la durée du stockage et de l'âge de la poule. Des œufs de poules Lohman en cages ont été inoculés avec une quantité connue de bactéries dans des conditions parfaitement définies. Des modifications caractéristiques du temps de relaxation sont observées pour des niveaux > 10^7 - 10^8 c.f.u./g d'œuf. Elles correspondent généralement à des altérations optiques et olfactives du contenu de l'œuf. La résistance des œufs contre la colonisation bactérienne diminue avec la durée du temps de stockage avant inoculation. Les œufs de poules jeunes ont une meilleure défense que les œufs de poules âgées. Le pourcentage d'œuf NMR-positif augmente lorsque les unités haugh sont plus basses.

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Introduction

LR ^{1}H NMR may be used for the analysis of large food stuff samples (Capozzi *et al.* 1999) and has a potential for application in the quality control of shell eggs (Schwägele *et al.* 2001). It also allows the detection of microbially spoiled eggs (Kröckel *et al.* 2001) and thus offers a possibility for studying microbial growth in intact eggs without the need for destroying the eggs. The present study was performed to identify possible correlations between egg quality parameters and microbial growth in eggs.

Material and Methods

Standardized eggs from caged hens (Lohmann Brown) at 26, 31, 38 and 46 weeks of age were obtained one day after laying and stored at 20 °C and 60 % r.h. for 1, 8, 15 and 22 days before and up to 9 days after inoculation with a pool of washed bacterial cells consisting of *Serratia marcescens* BAFF-SM19*, Salmonella* (6-strain pool of *S.* Enteritidis, one strain each of *S.* Senftenberg and *S.* Typhimurium) *Pseudomonas aeruginosa* BAFF-P15 and *Staphylococcus aureus* DSM1104. Bacteria were suspended in 0.1 ml saline and injected into the albumen next to the shell at concentrations of about 10^3 (*Salmonella*), 10^2 (*Pseudomonas, Staphylococcus*) and 10 (Serratia) per egg. At each egg age as indicated above ten eggs were inoculated. The transversal relaxation times T2(1) and T2(2) of eggs were determined using a specially constructed '*minispec mq 10*' (Bruker). Eggs were judged 'NMR-positive' if their relaxation times deviated significantly from the negative controls by day 9 after inoculation. At this time positive eggs were broken and subjected to visual-olfactorial evaluation and bacterial count determinations. Haugh Units were determined from uninoculated eggs.

Results and Discussion

During storage of shell eggs at 20°C the NMR relaxation times T2(1) and T2(2) show characteristic changes (Schwägele et al., 2001). The T2(2) values decrease from 400-500 ms exponentially within the first 7 days after laying to 330-430 ms. Then, a slower linear decrease occurs, e.g. from 430 ms on day 7 to 360 ms on day 34 at a rate of about 2.6 ms per day. The average rate of decrease from day 1 to day 7 is about 3-4 times higher. Microorganisms influence this development if they reach sufficiently

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high levels within the egg to induce chemical changes which usually are accompanied by optical-olfactorial changes such as deviations in odour, colour and integrity of the egg content (Kröckel et al., 2001). In these cases the T2(2) values start to increase during storage and reach values which are 130-200 ms higher than without microorganisms. While the initial T2(2) values may differ between individual eggs by up to 100 ms the initial T2(1) values are in a rather narrow range of 23-26 ms. During storage there is almost no change. From day 1 after laying to day 2-3 there is a decrease by 1-2 ms. Within the following 15 days the values increase at maximum by 3 ms and remain at this level for the rest of the storage time. Microbially induced chemical changes within the egg are recognised by an increase of T2(1) at a significantly higher rate than in the absence of microrganisms and T2(1) values may reach 40-50 ms within 13-25 days. While in response to microbial activities the T2(1) values increase continuously the T2(2) values reach a maximum and then decrease again. Therefore, T2(1) is an important control when longer measuring intervals (e.g. once a week) are used.

For a given breed subjected to constant husbandry conditions and a defined feeding regime egg quality is affected by egg age and storage conditions. Aging effects, including the breakdown of hurdles important for the egg's defence against microbial growth, are expected to proceed more rapidly at 20 °C than e.g. at 5 °C.

The resistance of the eggs against microbial growth decreased with increasing storage time before inoculation (Fig. 1). In general, the percentage of NMR-positive eggs 9 days after inoculation was lower than the one of growth-positive eggs as evaluated by bacterial count determinations of the egg contents (Fig. 1). Eggs were judged 'growth positive' when the bacterial counts per gram egg content were at least 100 times higher than the inoculum. Increasing NMR values were only observed when bacterial counts were at least 10^7-10^8 c.f.u./g and when these bacteria through their metabolic activity induced changes in the chemical composition of the egg.

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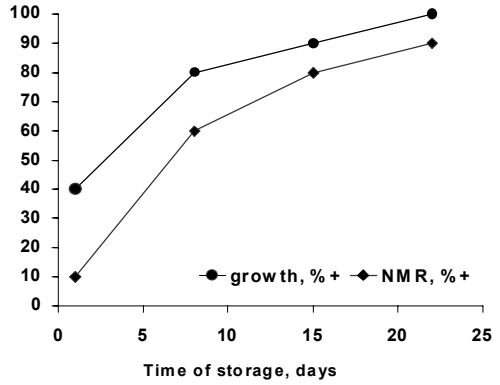

Fig. 1: Percentage of growth-positive and NMR-positive eggs stored at 20°C and 60 % r.h.. Eggs were stored for 1, 8, 15 and 22 days, inoculated and evaluated 9 days later.

In all cases microbially induced changes of the egg contents were due to *Serratia* and *Salmonella*. Upon breaking of the eggs these changes frequently correlated with optical-olfactorial deviations. When *Salmonella* spp. were dominant turbid albumen and a fishy smell were the characteristic deviations. The *Serratia* strain used for inoculation caused red discolourations and a fruity smell.
Eggs from younger hens (age 26 - 38 weeks) showed a higher resistance than the eggs from older hens (age 46 weeks) (Fig. 2). For very fresh eggs (day 1) hen age did not influence egg defence until living week 38. At present, it is unknown which egg components relevant as barriers for microbial growth are influenced by hen age.

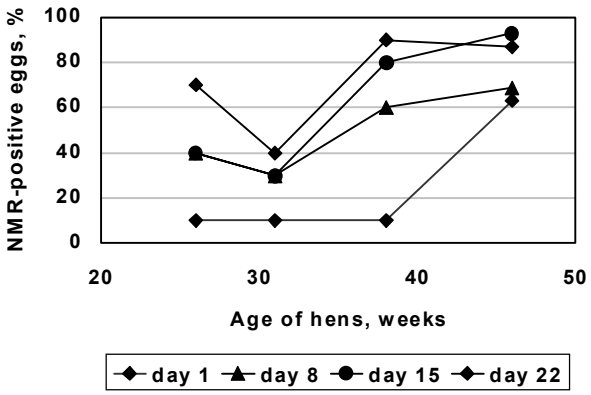

Fig. 2: Development of NMR-positive eggs for different hen ages (26 to 46 weeks) and egg ages (1 to 22 days).

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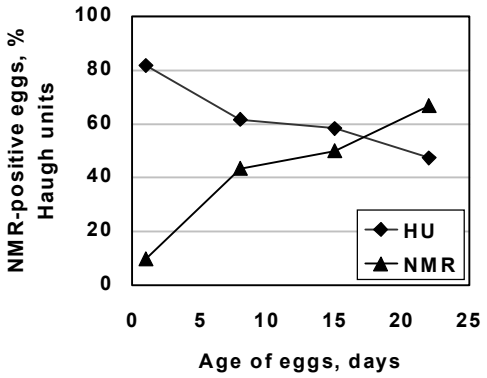

Fig. 3: Development of Haugh units (median of 90 eggs from hen ages 26, 31 and 38) and percentage of NMR-positive eggs (average from hen ages 26, 31 and 38).

Haugh units is a common measure related to albumen viscosity which both are decreasing with egg age (Haugh, 1937; Heath, 1977). The percentage of NMR-positive eggs increased with decreasing Haugh units (HU) (Fig. 3).
In summary, LR ^{1}H NMR spectroscopy is an interesting technique for studying microbiologically induced changes in hen eggs. According to our results egg defence against microbial growth is primarily dependent on the age of the eggs. However, they also indicate that younger layers produce eggs which are microbiologically more stabile than eggs from older layers.

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USING E-BEAM IRRADIATION TO IMPROVE POULTRY PRODUCT SAFETY AND SHELF-LIFE

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Keywords: Electron beam irradition, *Salmonella*, *Campylobacter*, Poultry quality

Abstract

Electron beam irradiation combined with aerobic or vacuum packaging was investigated as a means of extending the shelf-life of skinless, boneless breast fillets stored at 4 C. Boneless, skinless chicken breasts fillets were subjected to an electron beam irradiation dose of 0.8 kGy and stored under aerobic or vacuum packaged conditions for 42 days at 4 C. Electron beam irradiation completely eliminated coliforms and generic *E. coli* and the pathogenic organisms, *Salmonella* and *Campylobacter* in irradiated breast fillets. Vacuum packaging reduced the amount of lipid oxidation that occurred regardless of irradiation. Non irradiated fillets had a shelf life of about 14 days whereas the shelf-life of the irradiated fillets was around 42 days at refrigerated storage temperatures.

Résumé

L'ionisation combinée avec le conditionnement sous air ou sous vide a été évaluée pour la conservation de filets de poulets désossés sans peau. Les filets ont été traités par un faisceau d'électrons à 0,8 kGy et stockés sous air ou sous vide 42 jours à 4°C. L'ionisation permet l'élimination totale des coliformes, d'E. Coli, de salmonelle et de campylobacter. Le conditionnement sous vide permet la diminution de l'oxydation des lipides indépendamment de l'ionisation. Les filets non ionisés ont une durée de vie de 14 jours, contre 42 jours pour les filets ionisés aux températures de réfrigération.

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Introduction

Use of irradiation to improve safety of meat and poultry has been studied for over 40 years, but irradiation has not been favored by consumers because of misconceptions associated with irradiation. However, since FDA approved the use of irradiation in meat and poultry, it is now being reevaluated more as a means for improving safety of these products (Frenzen et al., 2000; Federal Register, 1992). Gamma and electron beam irradiation are the most commonly used irradiation sources for food, but electron beam irradiation is thought to offer several advantages over gamma irradiation (Satin, 1996; Heath et al., 1990). These advantages include higher dose rate capability, accelerators used to generate electron beams can be easily switched on and off, and more importantly, it allows application in a bi-directional manner (top and bottom of product). Application in a bi-directional is thought to result in more uniform application providing more effective elimination of bacteria on product surfaces. Currently in the US, electron beam irradiation (Surebeam) is being used commercially by several beef companies to ensure safer beef products for consumers.

Previous research has shown that electron beam irradiation is effective in reducing microbial contamination in poultry products and extending the shelf-life of poultry (Heath et al. 1990 and Shamsuzzaman et al. 1995). Irradiation treatment has been shown to affect product quality by increasing lipid oxidation and altering color and texture of meat (Shamsuzzanan et al. 1995 and Lewis et al., 2002). Because, the type of irradiation used, the doseage applied, and packaging can effect product quality, it was important to determine the effect of combining low levels of electron beam irradiation with different packaging systems that would potentially retard lipid oxidation and improve product quality. Therefore, the purpose of this research was to determine the effectiveness of electron beam irradiation at dose of 0.8 kGy in reducing microbial contamination in boneless, skinless chicken breasts.

Material and Methods

Irradiation Treatment

Fresh boneless, skinless chicken breasts that were packaged four fillets per polystyrene tray and shrink-wrapped with polyethylene overwrap were obtained from a commercial processing plant. Breast fillets were randomly divided into groups of non-irradiated and irradiated. Vacuum packaging was done using a commercial Multivac system.

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At the irradiation facility the next day, a commercial scale electron beam accelerator was used to apply an irradiation dose of 0.8 kGy (10 MeV, 50 kW) in a bi-directional manner. Amount of radiation received was measured by using Far West Technology dosimeters, which were placed in standard dosimeter plates and passed through the irradiation system. Experiments were duplicated with a total of 120 packages of breast fillets being used for microbial analysis and 120 packages used for sensory evaluation.

Microbioligical Evaluation

After color determination, the fillets were tested for microbial levels. Total aerobic plate counts and psychrotroph counts were determined using total aerobic petrifilm plates in duplicate. Plates were incubated at 37 C for 48 h for total aerobic plate microorganisms and 10 d at 4 C for detecting psychrotrophs.

For detection of *Salmonella*, samples were enriched in tetrathionate broth and plated on the XLT4 agar. Colonies were screened on triple sugar iron agar (TSI) slants and were serologically confirmed with *Salmonella* O antiserum poly A-I and Vi test kits.

For *Campylobacter* detection, samples were enriched with bolton broth and buffered peptone water. Defibrinated horse blood was also added to the broth. Samples were incubated in anaerobic gas chambers at 42 C for 32 h along with atmosphere generating systems (ascorbic acid) and. After 32 h, the samples were streaked on modified CCDA (MCCDA) plates and incubated at 42 C for 32 h. Typical colonies were confirmed with a latex agglutination kit specific for *Campylobacter*.

Sensory Evaluation.

Sensory evaluation was conducted biweekly using untrained panelists in the Department of Food Science and Technology. The panelists were served samples from irradiated groups up to Day 42 and up to 14 days for controls. The panelists were served one cooked sample at a time in a pre-determined random order and asked to rate each sample using a modified 9-point Hedonic scale. The Hedonic scale included the attributes of appearance, flavor, and overall acceptability (like extremely to dislike extremely) and texture (extremely moist to extremely dry). Raw product color was determined using a Minolta colorimeter. The degree of oxidative deterioration of the lipids in the fillets was measured using the TBARS analysis method of Witte et al. (1970).

Results and discussion

Electron beam irradiation dose of 0.8 kGy was effective in reducing population levels for total aerobic bacteria and psychrotrophs (Figure 1). As storage time of the breast fillets increased, there was a significant (P <0.05) increase in aerobic and psychotroph population levels for both non-irradiated and irradiated samples. However, the increase in population levels was lower (P <0.05) in irradiated samples. Heath et al.

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(1990) found that an electron beam irradiation dose as low as 1.0 kGy was effective in reducing total number of aerobic organisms by 2 to 3 logs in whole breasts and thighs. Results from a study by Luschsinger et al. (1996) indicated that an electron beam dose of 2.5 kGy resulted in a 4-5 log reduction of aerobic plate counts in boneless pork chops.

In the current study, we also determined the that 0.8 kGy eliminated *Salmonella* and *Campylobacter* (Figure 2*)*. Only the percentage of positive samples will be reported for the current study because MPN was below detection levels for these microorganisms. Heath et al. (1990) showed that an electron beam irradiation dose of 1.0 kGy was also effective in eliminating *Salmonella* from chicken meat. Lewis et al. (2002) indicated that an electron beam dose of 1.0 kGy was effective in completely eliminating both *Salmonella* and *Campylobacter* in chicken breast fillets. Our study indicates the effective level of irradiation to eliminate these pathogens can be reduced even further to 0.8 kGy.

Applying an electron beam irradiation dose of 0.8 kGy is an effective means of eliminating bacteria from breast fillets and extending product shelf-life without signficantly altering product acceptability. Specifically, irradiation increased product redness on raw product, but this effect was not detected in cooked fillets (data not shown). Sensory evaluation indicated that electron beam irradiation had no major effects on appearance, texture, flavor, and overall acceptability of the breast fillets (Table 1). However, as storage time increased, irradiated samples could not be compared to controls be of the high microbial loads present on control samples. Perhaps including a fresh non-irradiated control at each time point would have provided a means for panelists to distinguish quality differences between samples at a given time point.

Based on combined microbiological and sensory evaluation results, shelf-life of breast fillets were increased from 14 days to 42 days due to irradiation treatment .

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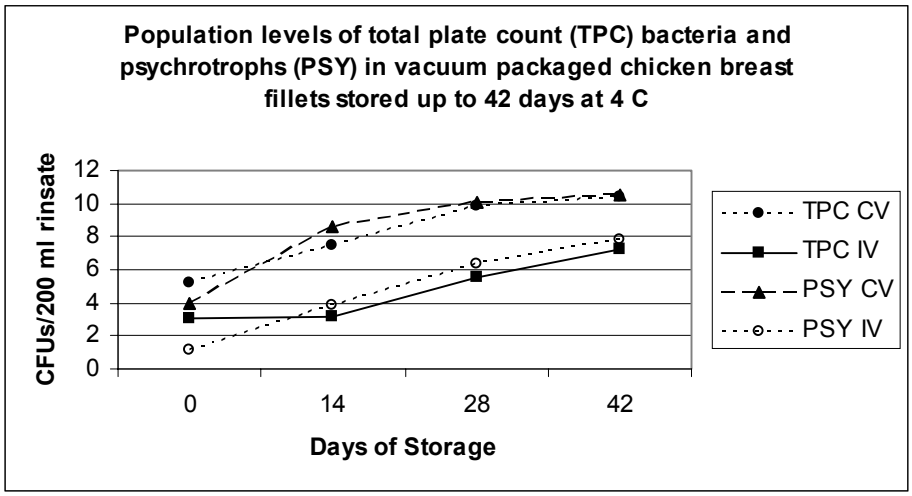

Figure 1. Total plate counts and psychrotrophs during storage of irradiated –vacuum packed poultry breast fillets.

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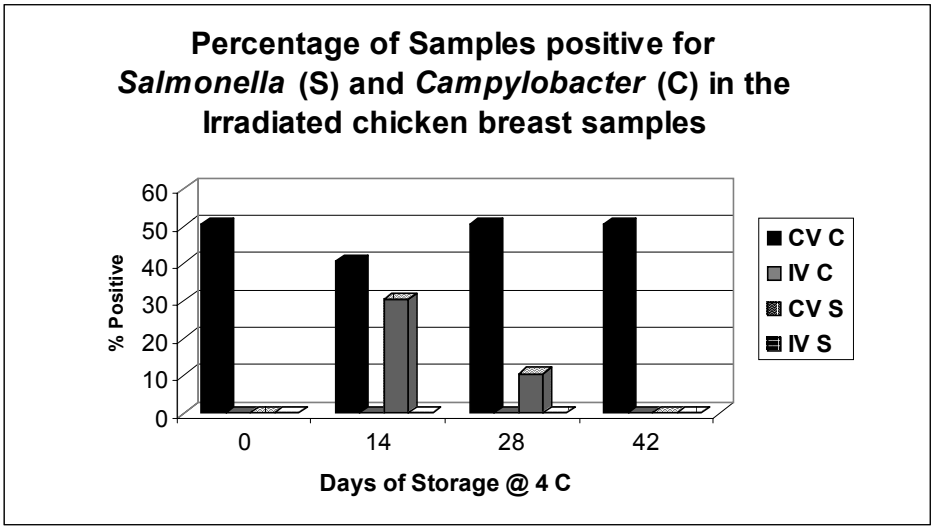

Figure 2. Percentage samples positive for *Salmonella* and *Camplybacter*

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Table 1. Hedonic ratings for quality attributes of non-irradiated and irradiated chicken breast fillets stored up to 42 days at 4 C

Quality		Days at 4 C			
Attribute	Samples	0	14	28	42
Appearance*	CV	6.65^{aA}	6.33^{aA}	----	----
	IV	6.58^{aA}	6.52^{aA}	7.15^{aB}	7.00^{aAB}
Texture**	CV	6.32^{abAB}	6.05^{aB}	----	----
	IV	5.83^{bA}	5.63^{aA}	6.25^{aAB}	6.43^{bAB}
Flavor*	CV	6.83^{aA}	6.57^{aA}	----	----
	IV	6.60^{aA}	6.42^{aA}	6.60^{aA}	6.85^{bA}
Overall Acceptability*	CV	6.78^{aA}	6.37^{aA}	----	----
	IV	6.53^{aAB}	6.10^{aB}	6.52^{aAB}	6.82^{bA}

$^{a-b}$Mean values within the same column with the same superscript are not significantly different ($P<0.05$).
$^{A-B}$Mean values within the same row with the same superscript are not significantly different ($P<0.05$).
CV = Control/Vacuum Packaging; IV = Irradiated/Vacuum Packaging.
---- Control samples were not tested after Day 14.
*Where 9 = like extremely, 5 = neither like nor dislike, 1= dislike extremely.
**Where 9 = extremely moist, 5 = neither moist nor dry, 1 = extremely dry.

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EVALUATION OF VARIOUS COOKING METHODS TO ENSURE THE SAFETY OF EGG CONSUMPTION

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Keywords: Egg Safety, Cooking Temperature

Abstract

Until 1996 *Salmonella* Enteriditis (SE) was the most common cause of all foodborne disease outbreaks in the US. Of these cases, undercooked eggs were the most common source of infection. Various agencies have set time and temperature recommendations for cooking all parts of the egg to 63°C for 15 seconds in order to kill SE. Unfortunately, consumer preferences do not always agree with these recommendations. This study was designed to answer consumer's questions about the safety of eating eggs cooked by various methods. Three sizes of non-inoculated eggs were cooked using poaching, boiling, scrambled, sunny side up, and over easy cooking methods. To ensure the safety of consumers eating eggs, results indicated that eggs should be boiled, poached or scrambled.

Résumé

Salmonella Enteriditis (SE) est la première cause de toxi-infections alimentaires aux Etats-Unis depuis 1996. La consommation d'œufs insuffisamment cuits en est la principale origine. Différents instituts ont émis des recommandations de cuisson des œufs à 63°C pendant 15 secondes, afin de détruire SE. Malheureusement, le goût des consommateurs n'est pas toujours en adéquation avec ces recommandations. Cette étude a été entreprise, afin d'évaluer le niveau de sécurité des œufs cuits par différentes méthodes. Trois calibres d'œufs non inoculés ont été cuits par différentes méthodes. Des œufs pochés, brouillés, durs, sur le plat ont été préparés et d'autres méthodes de cuisson couramment utilisées ont été évaluées. Afin d'assurer la sécurité des consommateurs, les œufs doivent être servis pochés, durs ou brouillés.

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Introduction

From 1988 to 1996, *Salmonella* Enteriditis (SE) was the most common cause of all foodborne disease outbreaks in the United States, with undercooked eggs being the primary source of infection. After years of increases in reported cases, epidemiological studies suggest that SE infections are decreasing (Olson *et al*., 2001). While the implementation of new quality and safety programs by the nation's egg industry appears to be having positive effects, SE remains an important health concern. There are two pathways of contamination of an egg by SE. It is possible for the egg to be infected during formation or by translocation of the organism through the shell. Investigators have demonstrated that ovarian infection is the most likely route of infection (Gast and Beard, 1992). Subsequent growth of the bacteria is predominantly regulated by temperature and storage time. If these infected eggs are subjected to temperature abuse, the SE can grow to high enough concentrations that some cells could survive an inadequate cooking process. The contents of an infected egg typically show no changes in appearance or odor that would alert the consumer. While there is a possibility of bacterial contamination in retail eggs, proper cooking can eliminate the risk of foodborne illness from SE.

Consumer preferences do not always agree with the recommendations of cooking eggs to complete firmness. This study was designed to investigate the time-temperature relationship of yolk and albumen consistencies to explore the possibility of cooking eggs to a specific temperature or visual endpoint that would ensure the destruction of SE while leaving the yolk less than solid. Our objective was to relate time and temperature combinations that will destroy SE to cooking procedures that would be appropriate and easy to follow.

Heat coagulation of egg components does not occur instantaneously at a given temperature. Turbidity develops and an increased viscosity of albumen is seen at 57°C (135°F). Coagulation begins at about 62°C (144°F) for albumen and 65°C (149°F) for yolk. The albumen ceases to flow at about 65°C (149°F) and the yolk at 70°C (158°F). Whole egg coagulates between 62°C (144°F) and 70°C (158°F) and toughens extensively at 80°C (176°F). Temperatures required for coagulation are elevated by dilution, as seen when liquid (water or milk) is added to eggs for scrambling (Yang and Baldwin, 1995). Research has shown that the coagulation properties are different between intact yolks and blended yolk (Woodward and Cotterill, 1987).

Material and Methods

Freshly laid, washed and sized shell eggs were obtained from the Piedmont Research Station, Salisbury, N.C. They were candled to detect the presence of defects and

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subsequently stored at 4°C prior to use. Time / temperature profiles of the consistency and visual appearance of egg yolk and albumen were examined during the application of five cooking methods: poaching, boiling (hard and soft cooked), scrambling, sunny-side-up and over-easy. Three egg sizes were used to analyze the relationship of area to heating rate and heat penetration; U.S. Grade A medium (47-54 g), large (54-61 g) and extra large (61–68 g). Individual egg weights were recorded and the rate of heat penetration during cooking was determined on six replicate eggs for each of the three sizes. Digital photographs were taken to aid in visual descriptions.

Thermal Process Evaluation:

A procedure outlined by Beloian and Schlosser (1963) and Patashnik (1953) was utilized to evaluate the lethality of the cooking treatments. These calculations were based on the thermal death time (TDT) characteristics and z value of 4.33 reported by Schuman and Sheldon (1997). The thermal process was estimated by converting cooking temperatures recorded at 0.5 minute equal intervals into equivalent minutes at 60°C (140°F) using the formula: $F/t = 1/(\log^{-1}((60-T)/z))$

Where F/ t = the ratio of time in minutes (F) required to destroy an organism at a selected base temperature (60°C), to the time in minutes (t) required to destroy it at a given temperature. The total lethality of the cooking procedure was derived by a summation of F/ t values. The equivalent time at 60°C is estimated by multiplying the $\sum F/t$ by the equal time interval (0.5 minutes) that the readings were taken. An optimum process time was set by calculating the 5 log cycle reduction in SE recommended by USDA shell egg pasteurization requirements using the D value (0.28 minutes at 60°C, Schuman *et al*., 1997) of stationary phase *Salmonella* spp. in yolk. This resulted in a value of 1.4 total minutes at 60°C (140°F). The estimated total lethality of each cooking process was then compared to this value. These calculations are using a published z value and seem to correspond well with the FDA recommendation of cooking the egg to 63°C (145°F) for 15 seconds.

Results and discussion

During cooking processes, temperature and appearance of the eggs were monitored and documented. Heating curves shown are for the egg in each set with the slowest heating rate. All eggs were prepared directly from refrigerated storage (4°C). Hard and soft cooked eggs in this study started at a slightly higher initial temperature due to the time required for thermocouple insertion.

Hard and Soft Cooked:

Boiling produced an acceptable hard cooked egg with the typical light yellow color, mealy texture and reached temperatures well in excess of those required to destroy any Salmonella present.

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Scrambled:
The temperature of the egg mixtures with initial temperatures of 12-14°C (54-57°F) rose quickly during the scrambling process. After 1 minute, the coolest surface temperature recorded was 48°C (118°F) and after 2 minutes, even the most liquid portions had reached a temperature of 67°C (153°F). Three minutes produced softly coagulated curds with no visible liquid and final temperatures of 74 – 80°C (165-176°F), which could be considered safe.

Poached:
The heating curves for poached eggs, indicate cooking times ranging from 6 to 7 minutes to reach an acceptable temperature of 63°C (145°F). When an internal temperature of 63°C is reached, the albumen is soft set and the yolk has turned dark yellow with about half of the volume gelled and the rest liquid. If the egg is held 8 minutes, minimum internal temperature is approximately 78°C (173°F) and the yolk has no liquid portion and the outer sections are starting to turn light yellow as seen in a typical hard cooked egg.

Sunny-side-up:
Eggs cooked sunny-side-up, uncovered and without basting, do not reach a safe internal temperature within an acceptable time frame.

Over-easy:
Monitoring the temperature of the cold spot in over-easy eggs proved to be very difficult. The cold spot in the yolk is constantly moving. The eggs were fried five minutes on one side prior to turning. During the initial five minutes, the heating curves are the same as seen in eggs cooked sunny-side-up. When turned, the temperature of the previously heated areas, that are now on top, start to cool. Precise recommendations would be difficult to make based on these data. Typical consumers fry eggs between 1 to 5 minutes, which does not provide adequate heat treatment.

Under normal conditions, it is not possible to cook an intact yolk to an over-all temperature of 63°C. When the internal temperature has reached 63°C, the majority of the yolk is at a much higher temperature and has solidified. Recommending specific minimum cooking times or temperatures to prepare yolks that are still liquid may not be feasible. Many factors can influence the heating rate for eggs. Initial temperature of the egg, cooking equipment, ratio of water to eggs, and even altitude, which influences boiling temperature, could be factors during cooking. In preliminary trials, a ceramic glass, solid surface cook-top range was used. This range proved to be slower to heat than most conventional electric or gas ranges, taking as much as twice the time to bring samples to temperature. In addition, recommendations state to bring water and eggs to a “boil”. There are wide ranges of times and water temperatures that consumers could visually consider to be “boiling”. Even egg freshness or quality determines albumen and yolk height and the amount of spreading at breakout. These geometric parameters could affect heating rates during frying or poaching.

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Based on the results of this study, the recommended minimum temperature of 63°C for 15 seconds will provide adequate heat treatment with a wide margin of safety for the egg consumer. However, the practical measurement or description of a visual cue to this temperature may not be feasible. If consumers demand a yolk that is less than solid, soft cooking and poaching seem to allow for the most even heat penetration that would result in a yolk that is at least partially liquid when the cold spot has reached 63°C. Fried eggs should definitely be cooked on both sides, basted or in a covered pan.

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KEYWORDS

This classification is non exhaustive as it is based on key words provided by the authors and do not included those present in the abstract or text

XVIth European Symposium on the Quality of Poultry Meat
&
Xth European Symposium on the Quality of Eggs and Egg Products

September 23rd – 26th, 2003

Saint-Brieuc
France

Proceedings

Volume II : Quality of Poultry Meat

Volume I : New European regulations and their impact - Hygiene and microbiology of poultry production - Evaluation and control of risks in poultry production
Volume II : Quality of Poultry Meat
Volume III : Quality of Eggs and Egg Products

Realisation of proceedings : Geneviève Clément and Marie-Pierre Rouxel, ISPAIA

Introduction

This volume contains all the manuscripts of the reviews, oral communications and posters presented during the XVIth European Symposium on the Quality of Poultry Meat held in conjunction with the Xth European Symposium on the Quality of Eggs and Egg Products in Saint Brieuc – Ploufragan, France. It is additional to the volume devoted to egg quality sessions and to that containing the economical and hygienic quality sessions.

How muscular structure and metabolism can influence directly meat properties? Is it possible to control by mean of selection or molecular genetic approach these mechanisms? The session four will try to bring answers to these questions. The poultry nutrition still influences largely carcass and meat quality, particularly the lipid fraction. Moreover, with the increasing development of processed products the susceptibility to oxidation phenomena still remains a problem. Therefore, the session five will concern the nutritional effect on carcass and meat quality. Finally, meat quality can be also determined by the rearing conditions, the events occurring during catching and transporting the animals from the farm to the slaughterhouse and the slaughter, cold storage and processing technologies. These aspects will be developed in the session six.

I wish you a successful symposium and an enjoyable stay in France.

E. BAEZA
INRA-WPSA

Introduction

Ce volume contient tous les textes des revues, communications et posters presentés au cours du XVIème Symposium Européen sur la Qualité des Viandes de Volailles organisé en parallèle du Xème Symposium Européen sur la Qualité des œufs et des Ovoproduits à Saint Brieuc – Ploufragan, France. Il complète les volumes dédiés aux sessions sur la qualité de l'œuf et aux sessions concernant les aspects économiques et sanitaires.

Comment la structure et le métabolisme musculaire peuvent-ils influencer directement les caractéristiques de la viande ? Est-il possible de contrôler par la sélection ou la génétique moléculaire ces mécanismes ? La session quatre essaiera d'apporter des réponses à ces questions. L'alimentation des volailles influence largement la qualité des carcasses et de la viande, en particulier la fraction lipidique. De plus, avec le développement des produits transformés, la sensibilité aux phénomènes d'oxydation reste toujours d'actualité. La session cinq concernera donc les effets de l'alimentation des volailles sur la qualité des carcasses et de la viande. Enfin, la qualité de la viande peut aussi être déterminée par les conditions d'élevage, d'attrapage et de transport des volailles de la ferme à l'abattoir et par les technologies de l'abattage, de la conservation et de la transformation. Ces aspects seront développés dans la session six.

J'espère que vous serez intéressés par ce symposium et je vous souhaite un agréable séjour en France.

E. BAEZA
INRA-WPSA

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Review

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Session 4
Muscle characteristics, meat quality and processing abilities

ADVANCES IN POULTRY MEAT PROCESSING TECHNOLOGY.

B. Boutten

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Introduction

In recent years, the number of manufactured products made from chickens and turkeys has increased in France. It is estimated that processed chicken products, which represented 15 % of the French chicken market in 1999, will represent about 25 % in 2005 (Magdelaine and Philippot, 2000). A similar situation is observed in other European countries.

This significant increase was the result of new, convenient, brand-name products with added value and further processed products for food service. Moreover, the poultry industry has benefited from health-related concerns about animal fats and high protein concentration. Poultry breasts may have 24 % protein for 0.4 % fat. This led to an increase in the number of products available to consumers, such as nuggets, cooked products like brined cooked poultry breasts, poultry bacon and poultry frankfurters. Furthermore, there is a significant price difference in favour of poultry products by comparison to other meat products.

However, it is important to better evaluate the quality of products from the point of view of their brand images, as well as from the microbiological and technological points of view. For exemple, if the pH of the meat is high, it is easier to work without sodium tripolyphosphate.

This review focuses on the technological qualities, which may be affected by several factors (C. Berri *et al*., 2001). Indeed, quality depends on several economically important production steps such as breeding, transport of animals, slaughtering procedure and meat processing industry. Here, we considered only the factors associated with slaughtering procedure and transformation process.

Slaughtering procedure

Many factors during the life of the bird influence meat quality. These factors can be divided into two categories : those with a long-term effect and those with a short-term

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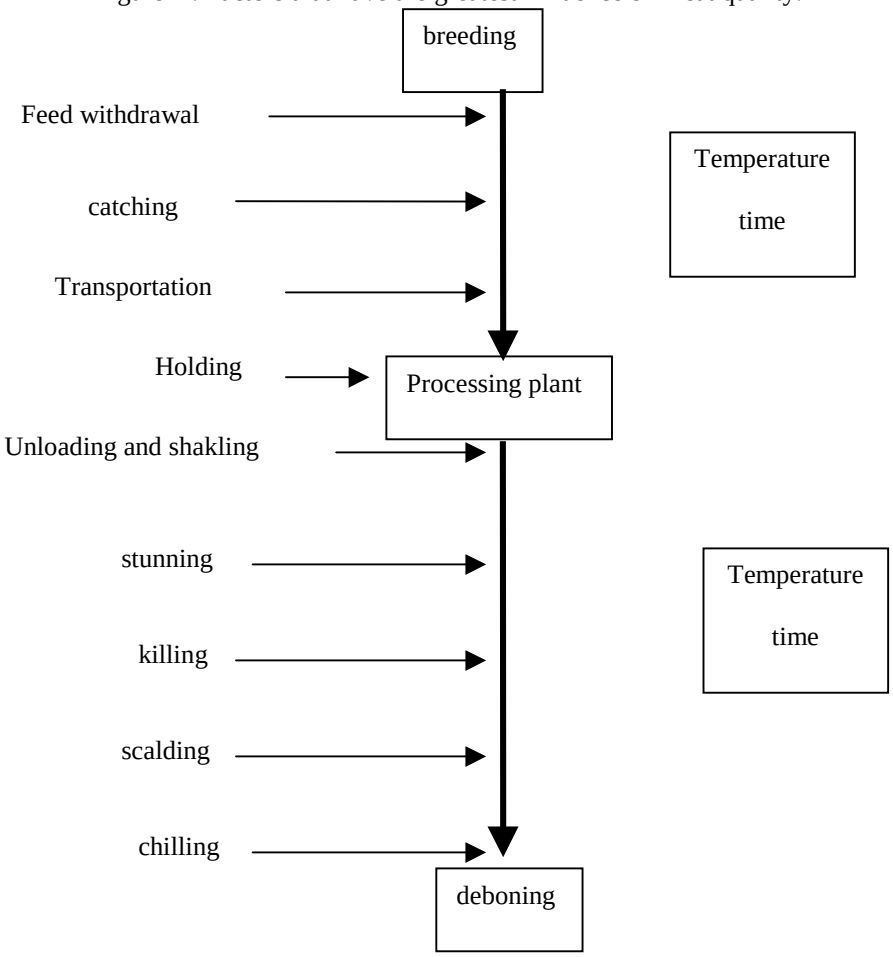
effect. Long-term factors are genetics, physiology, nutrition, management and disease, but are out of the scope of this study. Short-term factors affecting poultry meat quality are those that occur during the last 24 h of life. These factors are shown in figure 1.

Figure 1 : factors that have the greatest influence on meat quality.



The pre-slaughter factors that have the greatest effect on meat quality are feed withdrawal, transport, environmental temperature and stunning method. Pre-slaughter conditions affect the technological quality of meat by affecting cell glycogen concentration and metabolism.

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Before birds are caught, loaded, and transported to the processing plant, feed and water are removed to allow the emptying of the digestive tract. The removal of feed and/or water reduces the incidence of carcass fecal contamination that may occur during processing but also decreases the levels of muscle glycogen. The level of muscle glycogen at time of slaughter affects rigor mortis and ultimate pH (Smith, 2001).

When the bird dies, the cells continue their metabolism by using endogenous energy store, *i.e.* glycogen. Metabolism shifts from aerobic to anaerobic. The glycogen concentration affects the ATP production, that helps to regulate the interactions between the protein fibres involved in contraction and thus in *rigor mortis*.

The production of ATP, which is essential for muscle cells, is associated with the production of lactic acid. While lactic acid would be removed by the blood in the living animal, this compounds accumulates in the muscle cells of dead animals and causes the cell pH to decrease from about pH 7 to about 5.7. pH decrease depends on several parameters such as feed withdrawal , temperature and stress .

Also, changes in pH affect the activity of ATP-producing enzymes and thus the time taken to reach full rigor mortis and protein functionality.

The pH and time of rigor mortis affect the time when it is possible to cut and to debone. Cutting and deboning the muscle before rigor mortis may generate muscle contraction. The extent of muscle contraction is no longer limited by skeletal restraints, so the degree of shortening is greater for the free muscle.

It is generally considered that withdrawing feed between 8 to 12 hours for broilers and between 6 to 12 hours for turkeys is sufficient for good conditions (Sams *et al*., 1993) for the shear value as well as for the ultimate pH:.

The stunning method immobilises the animal, which allows efficient cutting of the blood vessels. Stunning reduces struggle and convulsions during bleeding and subsequently reduces associated carcass damage. However, it also modifies the post mortem metabolism of muscles.

Three methods are used for stunning (Fletcher, 1998):

- Electrical stunning in a water-bath under European conditions. Because of welfare reasons, broilers are stunned with current value near 90 mA, that allows the animal to become instantly unconscious and the heart failure to occur immediately.
- Electrical stunning in a water-bath under US conditions. Broilers are stunned with 10 –12 mA and low voltage, high frequency occurring in approximately 65 % of poultry slaughtering plants.
- Gas stunning, which has both animal welfare and carcass quality benefits.

The stunning method affects rigor mortis with changes in both the rate and extent of pH fall (Papinaho *et al*., 1996; Dickens *et al*., 1995). Electrical stunning under European conditions delays rigor mortis, without modifying the ultimate pH of meat, unlike electrical stunning in US conditions and gas stunning. The higher the intensity

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and frequency, the slower rigor mortis occurs. Harsher conditions also result in higher incidences of haemorrhaging and broken bones.
It is difficult to separate the stunning method from chilling conditions. Gas stunning is also associated with quick chilling, because pH decreases rapidly and rigor mortis occurs rapidly in association with a rapid decrease in temperature. This makes it possible to harvest the meat rapidly without cold shortening.
In European conditions, electrical stunning is not associated with hard chilling if the meat is harvested rapidly (2h30 for example for broilers). Beyond this point, cold shortening may be observed and it is necessary to wait 4 hours before deboning (Lesiak *et al*., 1997).
The relationships between rate of pH fall, ultimate pH and technological behaviour of meat are complex and influenced by the temperature of meat during pH change and by chilling. Simultaneous changes in the pH and temperature of muscle affect protein denaturation, the capacity of proteins to bind water and the technological behaviour of the meat (Santé *et al*., 1997).

On-line evaluation of poultry meat

We will concentrate on the on-line evaluation method, which is the main method used in the poultry processing industry to measure pH and colour.

PH

The post mortem pH of meat is determined by the amount of lactic acid produced from glycogen during anaerobic glycolysis necessary to maintain the adenosine triphosphate (ATP) concentration. ATP depletion is the biochemical event responsible for the development of rigor mortis.
The complex relationship between protein denaturation and pH can be explained by the importance of the temperature of meat at this pH and changes in these two factors.
Sante *et al.* (1997) and Sosnicki (1995) demonstrated that proteins are denatured during rapid post-mortem glycolysis and deposited on the filaments in the myofibrils, forming a sarcoplasmic protein 'coating'. Recent gel electrophoresis and immunochemical staining of myofibrils showed that two main enzymes, creatine kinase and phosphorylase, co-precipitate with the myofibrilar fraction of muscle when post-mortem pH decreases quickly while muscles are still warm. Partial myosin denaturation by this sarcoplasmic protein coating appears to be responsible for the poor extractability of the myofibrillar proteins in pale, soft, exudative (PSE) pig muscle. This is probably also the case in PSE-like turkey breast muscle. This difficulty to extract protein is similar to the protein that has a low water-binding capacity during technological processing.

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In low pH conditions, water is less strongly bound to proteins (the electric charge of proteins is lower when they are near their isoelectric points), and moves from intracellular compartment to extracellular compartment. It creates more reflective surfaces and increases the reflection of incidental light, making the meat paler.
Due to its effect on respiration and water distribution, pH is strongly related to colour.
The pH is used as an industrial index of quality for pork (Jacquet *et al.*, 1984). It is more difficult to apply these tests to poultry products, because a pork ham weights between 8 and 12 kg whereas a chicken breast weights between 100 and 300g. Thus, pH evaluation has to be more than 50 times quicker in poultry. In turkeys where the breasts are heavier, measurement of pH is more feasible.
Another problem associated with evaluating the pH of poultry products is the time between slaughtering and deboning: in the case of pork, pH is measured between these two steps, which are commonly separated by 16 or 28 hours. However, in poultry these are commonly separated by 4 hours or less. The pH measured at this time is not the final pH and the correlation with meat quality is therefore weaker

Colour

Colour is an important quality attribute that influences consumer acceptance of many food products but also predicts the technological behaviour of meat. Colour defects of raw and cooked poultry meat have been a problem for the poultry industry for many years.
Colour and pH are closely related in poultry (Barbut, 1998; Wilkins *et al.*, 2000; Santé *et al.*, 1991, Qiao *et al.*, 2001).
Colour defects of raw poultry meat, such as PSE-like conditions, were first reported in turkey breast meat (McCurdy *et al.*, 1996) but have subsequently been reported in broiler breast meat (Barbut 1998; Woelfel *et al.*, 2001).
After slaughtering, respiration is gradually inhibited. In deep tissues, the amount of myoglobin is reduced. At the surface, the oxygen is fixed to the myoglobin (oxymyoglobin).
When the pH is high, muscle fibres use the available oxygen. This means that the amount of myoglobin at the surface is reduced.
A combination of low pH and high temperature leads to the partial denaturation of proteins, decreases the consumption of oxygen, thus facilitating the oxidation of myoglobin into metmyoglobin.
For processing industries, it is easier to determine the colour of poultry breasts, due to their anatomical simplicity. This simplicity allows a sorting speed adapted to industrial conditions. Sebastian *et al.* (2003) attempted to apply this technique to an industrial situation.

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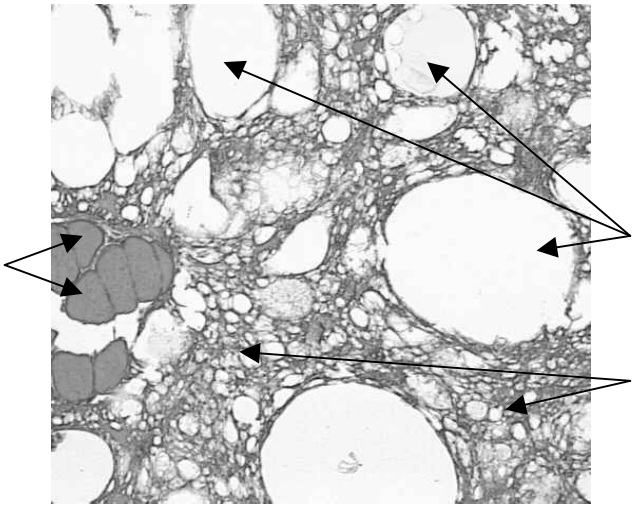
Implications for product

Two types of typical product have been considered: emulsified poultry products and brined, cooked poultry products.

Emulsified poultry products

Emulsified products typically contain about 15 to 18 % protein, 0 to 25 % fat and 50 to 80 % water. The products studied here included Frankfurter sausages. This product is the result of low-temperature emulsification of meat, fat and water principally before cooking. The final structure of the product is shown in figure 2. The functional properties of proteins in these poultry products can be divided into three categories: protein-water interactions, protein-fat interactions and protein-protein interaction.

Figure 2: frankfurter sausages, emulsion of meat, fat and water.



A large amount of the meat in this product is mechanically deboned poultry (MDP) (see figure 3). Several qualities can affect the chemical composition of the final product due to the presence of bone, cartilage and skin. These components may be found in raw materials but also in the finished product. The technical behaviour of denatured MDP (Figure 3A) differs from that of non-denatured MDP (Figure 3B).

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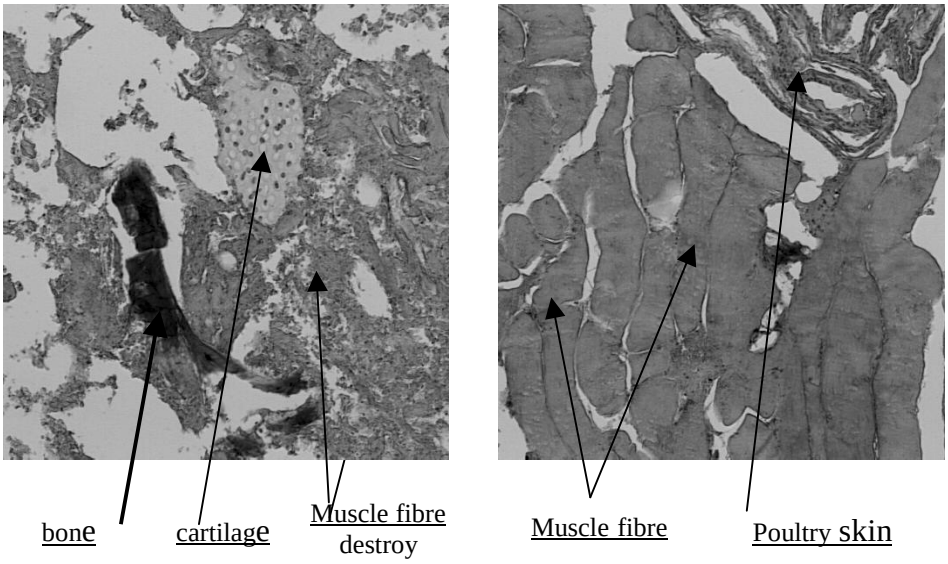
The quality of the meat affects the technological process used afterwards, because meat with high final pH binds water better. Water binding is lower at the isoelectric point of myosin and actin (near pH 5). If the pH of the product is high, it is easier to work without sodium tripolyphosphate. This solution is preferred by industrials due to the high quality of the resulting product.

Figure 3: Mechanically deboned poultry (A) with destroyed muscle fibre (B) without destroyed muscle fibre.



For the technologist, several products may improve meat quality due to their specific actions on meat. These products include salt and sodium polyphosphate.

Salt (NaCl or KCL) is always used. It helps to flavour the product, to lower water activity and to increase ionic strength, which delays microbial growth, with solubilisation of muscle proteins.

Salt breaks the electrostatic bonds between the protein thus opening up the protein structure and improving the binding of water.

When used at a concentration not exceeding 4%, salt increases the ionic strength and improves the solubilisation of the protein.

Polyphosphate has two effects. Firstly, it increases the pH slightly. Secondly, it binds the Ca2+ and opens up the actin-myosin structure of the protein. Thus, proteins bind water more easily (Belton *et al*., 1987).

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The other products that improve meat quality have less specific actions. For example, they:

- increase water holding capacity by exerting a gelling activity, e.g. gelatin, alginate and carrageenan,
- increase water holding capacity by binding, e.g. soy protein, milk protein, hydrolysed plant and animal proteins.
- make meat particle binding more muscle-like, e.g. transglutaminase and fibrimex.

Brined, cooked poultry products

New kinds of products develop such as brined, cooked poultry products that can be sent all over the world due to their longer shelf life or convenience culinary products.
The development of such products is made possible by:

- the technical progress of machinery,
- the development of additives or technology to compensate for deficiencies in the meat or to enhance its properties.
- The introduction of legislations to control the use of additives and to protect the consumer in most countries.

This situation allows profitability and ensures the quality of the products.
It is important to note that brined, cooked poultry products are the result of complex technology involving several steps. Every step affects the quality of the final product, for example:

- Cutting and trimming; these procedures are partially carried out manually due to the lack of appropriate machinery. It is necessary to eliminate the connective tissue and fat that give the final product an undesirable colour, but this also facilitates the solubilisation of proteins, improves the binding of muscles and prevents retraction during thermal treatment
- Injection; the curing of meat consists in the addition of a number of additives and ingredients that are essential for its colour and flavour. These, together with water, form the brine that is homogeneously injected into the meat. The percentage of brine injected is determined by the desired quality of the finished product, and all subsequent phases of the process depend on this factor**.** The homogeneous distribution of brine in the meat muscles influences not only the appearance of the slice (preventing the visibility of brine lines and irregularity of colour) but also the final yield, as brine ingredients responsible for proteins solubilisation will be received by all muscle fibres. This difference is illustrated in figure 4; in figure 4A the brine is correctly distributed between the muscles and allows good slicing, whereas in figure 4B the brine is not correctly distributed between the muscles, resulting in problems with consumer acceptability and slicing properties.

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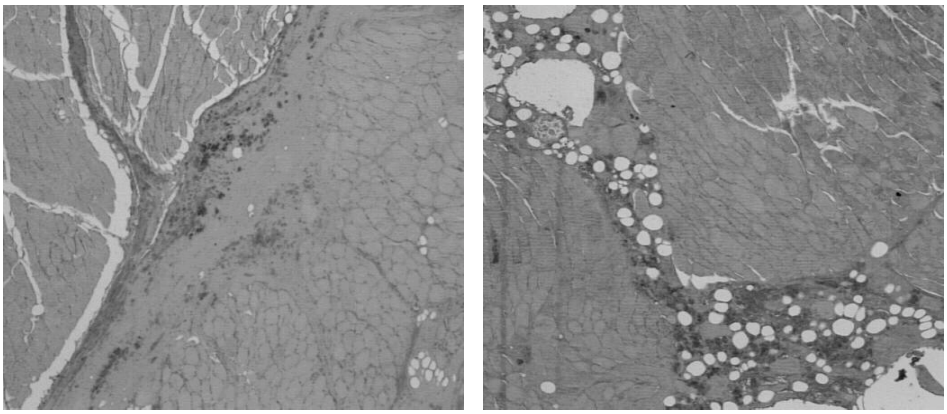
- tumbling, which is responsible for the binding of muscles and water holding capacity of the product. The muscular components responsible for these two characteristics are the myofibrillar proteins which, once extracted and solubilised, form the exudate (a kind of film on the surface of the meat) with a glue-like effect between the muscles. For water retention to occur, it is necessary that these proteins remain "open" so that water can penetrate them.
- Cooking results in the coagulation of muscle protein, the formation of characteristic flavours and aromas, the stabilisation of colour and the destruction of microorganisms
- Slicing in industrial conditions is very complex and generally explains the use of additives.

Figure 4: Two situations observed on exudate between muscle with a glue-like effect.



Brined, cooked poultry products are subject to regulations. There are a number of differences between standard and superior quality products. Standard quality products can contain binding agents: cellulose, protein and gelatin at a concentration of 2% and also ingredients with gelling function similar to carrageenan at a concentration of 0.5%. Nearly all the raw materials are authorised. Superior quality products can only contain 0.3 % of gelling agents (carrageenan for example). The regulations concerning the raw materials are stricter, and several industrials do not add any such ingredients. Therefore, the raw meat has to be of very good quality.

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Conclusions.

The increase of poultry manufactured products requires the quality of these products to be improved. Most countries have introduced legislation to control the use of additives and protect consumer.
The quality of these products requires a better control of meat quality, in order to make it possible to avoid using technological aids, and give to the consumer a better image of the product.
The control of meat quality requires knowledge about the principal factors affecting meat quality and the use of indicators of meat quality. The easiest way of improving meat quality is to improve pre-slaughtering procedures: feed withdrawal, transport, stunning, chilling. All these steps affect meat quality as well as pH and colour, which are commonly used as measurements of meat quality.
The most commonly used indicators of meat quality are currently: pH and colour. It is very difficult to use pH in chicken processing but it is easier in turkey processing. Colour appears to be the best predictor of the technological behaviour of brined cooked meats.
Technological and technical advances in machinery and auxiliary components make up the line, obtaining more versatility and automation. This has allowed the standardization of some processes and ensures that the quality of products remains constant.

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CONTENTS

MUSCLE FORMATION, GROWTH, AND FIBER DIVERSITY THEIR IMPLICATIONS FOR MEAT PRODUCTION

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Introduction

The developmental origins of muscle fibers in the chicken are well understood [1]. But how fibers of differing function emerge, how once formed muscle they grow, and how this understanding can lead to enhance quality of meat are not understood.
To place these unknown issues in perspective it is important to understand how muscle is formed. All the skeletal musculature of the chicken originates in the early chick embryo from the segmentally arranged somites [2, 3]. The somites are spheres of cells that are formed in pairs from head to tail along side the forming spinal cord. By the third day of embryonic development of the chicken all the somites that will form have appeared. By the third day of development nearly all the somites have become compartmentalized into a muscle-forming region and a bone-forming region [4]. This compartmentalization is orchestrated by signaling molecules that are released from adjacent tissue structures. The medial portion of the somite gives rise to the intrinsic back muscles (epaxial muscles) of the chicken while the lateral portion gives rise to all the muscles of the limbs, chest, and abdominal wall (hypaxial muscles) [5]. The bone-forming compartment produces the vertebrate, ribs, and scapula [6].

Tissue Interactions Establish Epaxial and Hypaxial Skeletal Muscle Cell Lineages.

The molecular signals that establish the muscle forming regions of the somite are produced in the neural tube, the notochord and the overlying skin (ectoderm) and other adjacent structures. Many of these signals that commit somite cells to a muscle fate have been identified [3]. The signals are proteins that diffuse from the tissue of origin to the cells of the somite. For example, both the neural tube and the surface ectoderm produce bone morphogenetic proteins (BMPs), while the neural tube also produces signals such as Wnt proteins and members of the hedgehog family of proteins [7-10]. These are proteins that interact with somite cells by combining with receptors on their cell membranes. The binding of these signaling proteins to their receptors on the

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surface of somite cells alters the internal metabolism of the somite cells activating genes required for muscle cell formation. While activation of many genes is require for a cell to differentiated into a muscle cell, the genes most important for commitment of cells to a muscle fate are call myogenic regulatory genes that encode proteins called myogenic regulatory factors (MRFs). The MRFs function as transcription factors within the nuclei of somite cells to activate a cascade of genes expressed in skeletal muscle.

Like the epaxial muscle, those somite cells that are the precursors of the hypaxial muscles of the legs and wings, require signals from the ectodermal (Wnts and BMPs) and from the lateral plate mesoderm [11, 12]. However, these precursor cells differ from those that form the intrinsic muscles of the back because they must migrate from the somite to the limb buds to form muscles of the wings and legs [13]. Formation of all muscles of the limbs depends on the migration of these muscle precursor cells from the somites to the limb buds that form in the early embryo. Without this migration wings and legs form with bones and tendons but no muscle. When the migratory precursor cells reach the limb bud they proliferate and then fuse with one another to form long muscle fibers with many cell nuclei typical of a muscle fiber.

The Origins of Muscle Fiber Types in the Embryo and Fetus.

While it is clear that muscles form in the back, wing or leg, there remains some controversy as to how fiber types and their patterning is established in these muscle forming regions. To adequately understand the mechanism involved it is important to understand that there are different types of precursor cells that form in the somite before they migrate to the limb [14]. All anatomic muscles found in the adult chicken have their origins in several waves of muscle fiber formation as development proceeds [1, 15]. The first fibers during embryonic development are designated primary fibers. The primary fibers in both birds and mammals are formed from the first population of myoblasts, called embryonic myoblasts that appear in muscle forming epaxial or hypaxial regions. The amount of muscle mass formed from the embryonic myoblasts is extremely small, so their role in muscle formation is to define the type, shape or location of a muscle, rather than to provide mass. On the other hand, much of the muscle mass that ultimately will form in the adult chicken is dependent upon the second wave of myogenesis first detected as the transition from embryonic to fetal development occurs (about 8 days of incubation). This second wave of fiber formation is designated as secondary fiber formation and contributes greatly to the number of fibers that will be found in the mature muscle. Secondary fibers (fetal fibers) have their origin in fetal myoblasts. The addition of most new fibers to anatomic muscles occurs during fetal develop by addition of fetal fibers particularly on

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the periphery of primitive limb muscles with the muscle fibers closest to the limb bones being those that are more likely to be of primary fiber origin.
The first fibers to form in the embryo are formed from embryonic myoblasts which are of two basic types – those that differentiate into muscle cells that express slow and fast isoforms of myosin heavy chain (MyHC) and those that produce only fast myosin heavy chains [16]. These myoblasts differ in the types of fibers they form and they appear to migrate into the limb at slightly different times in development. Fetal myoblasts differ from the embryonic myoblasts because they form only fast myosin expressing fibers, unless they are innervated by nerves. Thus the basic functional type of muscles that will form in different regions of the limbs is dependent upon the types of myoblasts from which the muscle forms and upon its pattern of innervation. Once the primary fibers are formed from embryonic myoblasts they become innervated and fetal myoblasts attach to their surfaces and begin to increase the number of fibers within the muscle and therefore the size of the muscle[17, 18]. Secondary fibers increase rapidly in number and nucleation, and subsequently diameter, and they come to be surrounded by their own basal lamina as they separate from the primary fiber on which they formed. It is this sequential formation that has lead to the concept that primary fibers form the scaffolding of the future muscle upon which secondary fibers form. One can postulate that primary fibers lay down the location and anatomic shape and fiber type of a muscle serving to organize all subsequent fiber formation.

Embryonic, Fetal, and Adult Myoblasts

All skeletal muscle fibers of developing muscles form by fusion of myoblasts with one another from distinct populations of myoblasts that populate growing muscles at specific times during muscle formation[19-21]. It is myoblast fusion that is responsible for the multinucleation of the fiber. As outlined above there are three categories of myoblasts, embryonic, fetal, and adult or satellite cells. Embryonic myoblasts differ from fetal and adult myoblasts in several respects. In birds, embryonic myoblasts form fibers that are short containing one or only a very few nuclei and that express specific isoforms of MyHC [22]. In chicken these differences in MyHC expression both *in vivo* and *in vitro* serve to define the fiber type content or pattern of the fully developed anatomic muscles of the limbs. What is clear is that in the bird the primary fibers primarily are responsible for the formation of slow type or type I fibers[16].
Fetal myoblasts appear near the end of the first week of development of the chick limb [20, 23]. These myoblasts differ from embryonic myoblasts because they form myotubes with many more nuclei than primary fibers – they are very long muscle fibers. In the bird, fibers formed from fetal myoblasts also differ from those formed from embryonic myoblasts in the isoforms of MyHC expressed and the influence of

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innervation on MyHC isoform expression within fibers they form. All fibers formed *in vitro* from fetal myoblast express fast isoforms of MyHC, whereas fibers formed from embryonic myoblasts can form fibers of differing MyHC expression. Also fetal myoblasts isolated from differ muscle anlagen form fibers that respond to innervation differently [24]. Those fetal myoblasts isolated from anlagen that are of slow fiber muscles (the red muscles or 'dark meat') will express slow as well as fast isoforms of MyHC upon innervated *in vitro*, whereas those formed from fetal myoblasts from fast muscle anlagen will not. Thus there appear to be differ types or lineages of embryonic myoblasts and fetal myoblasts in both avian and mammalian limbs based upon their time of appearance, the size of fibers formed, the isoforms of MyHC expressed, and the influence of innervation on the fiber type expressed.

Embryonic and Fetal Myoblasts Numbers and Fiber Formation

The numbers of myoblasts and therefore number of fibers (size of a muscle) is dependent on a number of factors [25]. Key among these is proliferation of myoblasts in response to growth factors produced in the tissues in which muscles are forming. Fibroblast growth factor family members (FGF) have key roles to control early myoblast number [26]. Also the insulin-like growth factors (IGF) are important [27]. There appears to be both growth promoting influences represent by FGF and IGF and growth retarding mechanisms controlled by members of the TGF-beta family of signaling molecules. It is the interactions of positive and negative signals that modulate muscle size initially by controlling myoblast cell number [28].
Myostatin, a member of the TGF-beta superfamily, is a negative regulator of fetal and later stages myogenesis [25]. Myostatin acts as an inhibitor of myogenesis. In mice with a knockout of the myostatin gene and in cattle with a mutation in this gene (Belgian Blue and Piedmontese cattle), muscle fibers are hypertrophic and perhaps more plentiful [29, 30]. It is proposed that animals that lack myostatin have more myoblasts and thus can form more fibers. It can be postulated that the control of fiber formation during fetal life involves the orchestration of myostatin, other TGF family members, FGF, and IGF family members to modulate the fetal myoblast number and secondary fiber formation and size [28, 31].

Muscle fiber Patterning

In the chicken the there are distinct patterns of fiber distribution that characterize muscles. For example, the pectoral muscle of the white leghorn, contains only fast

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muscle fibers (type II fibers), whereas the anterior latissimus dorsi – the muscle that keeps the wing against the body, is composed of only type I fibers [32]. Other muscles are composed of varying mixtures of fibers that express different myosin [33, 34]. The pattern of distribution of these fibers within each anatomic muscle appears in fetal development and persists through out the life of the bird. These patterns are quite precise and organized. What determines these patterns is not known. We know from studying quail that the pattern in the pectoral muscle is determined prior to the myoblasts migrating from the somite to form the pectoral muscle. This was demonstrated by grafting quail somites into chick embryos [32]. The quail pectoral muscle differs from the chicken because both fast and slow myosin-expressing fibers are found within the quail. When chickens are formed from embryos into which quail somites have been engrafted, the pectoral muscle formed the quail pattern of fiber distribution.

Current thinking suggests that the patterning of muscle fibers within chicken muscles originates early in development independent of innervation. Thus the pattern is determined by the cells that lead to muscle formation. The forces that carry out this function could be those that are contributed by the cellular environment into which the myoblasts migrate, though as indicated above in the quail-chicken chimera experiments, the migrating cells themselves are important in patterning. The reason it is clear that pattern is not dependent on the nervous system is because limbs form normal muscles and fiber patterning in embryos lacking a nervous system. However, once formed in fetal life, the pattern is nerve or innervation dependent, because denervation or blocking of function innervation at this stage alters the pattern of myosin expression and the pattern that is detected.

Thus muscle fiber type and distribution has a nerve-dependent and a nerve-independent phase. Both phases are important in the final formation and functional plan of each muscle.

Innervation and muscle fiber number and size

It is well demonstrated than for secondary fiber formation is independent of innervation in primary fibers [35-38]. Because the normal increase in numbers of secondary fibers is diminished if innervation does not occur, we know that a relationship exists between innervation and fetal myoblast proliferation. Thus increases in secondary fiber size are heavily dependent upon sustained innervation of primary and secondary fibers [34]. Therefore, innervation is a key determinate of muscle size and is crucial in maintaining the pattern of MyHC expression within specific fiber types [16].

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Muscle hypertrophy and regeneration

There is a marked increase in muscle size that begins in neonatal life and continues into adulthood. This increase results from an increase in the size of existing fibers, called skeletal muscle hypertrophy. Unless injury occurs, birds do not form many new fibers in the neonatal period [39]. The fetal period is the principal formative period of muscle fiber number and the neonatal and adult periods are those of hypertrophy and repair. Thus, in most animals, muscle mass increase beyond the postnatal period is dependent upon protein synthesis and not new fiber formation.

The BUT-T9 strain of turkey is characterized by high body weight (HW) and fast growth rate compared to the lightweight (LW) or slow growth bird [40]. At hatching HW body weight and muscle fiber number were almost identical to those of LW birds. However, at 15 weeks, the HW birds weighed twice as much as the LW birds and had enlarged muscle fibers (increased over 8 time to hatching stage). More significantly HW birds contained almost twice the number of muscle fibers present at hatching. Recent observations suggest that variations in the postnatal muscle growth pattern between HW and LW strains may be related to a difference in the capacity of their satellite cells to proliferate. Thus there are mechanisms that can increase fibers number and size leading to increased meat production which most likely results from the formation of new fibers from satellite cells in the postnatal period.

Hypertrophy in the postnatal period in most vertebrates is under the control of mechanical processes of muscle stretch, and activity that are transduced by a number of signaling molecules. Prominent among these molecules are the IGF family [41, 42], FGF [43], interleukin-6, and leukemia inhibitory factor (LIF) [44], and TGF family members including myostatin [45] and the production of nitric oxide [46]. Hypertrophy is associated with adult myoblast (satellite) activation in mature fibers. Both hepatocyte growth factor (HGF) [47], and c-met, its receptor, are required for adult myoblast activation. Transgenic mice that overexpress the *IGF-I* gene demonstrate marked hypertrophy of muscle fibers without elevation of blood levels of IGF-I supporting the contention that the effect of IGF-I can be an auto- or paracrine rather than just an endocrine effect [48].

Some of the factors that initiate hypertrophy have mechanisms in common. In response to mechanical load calcium ions are sensed by calcineurin which couples the mechanical work to muscle gene expression. Because calcineurin, a calcium-regulated phosphatase, has also been implicated in signaling related to overload hypertrophy of the heart, calcineurin has been pursued as an intermediate in the transducing pathway in skeletal muscle hypertrophy [49]. Activated calcineurin mimics the effects of IGF-I on cultured myogenic cells, whereas expression of a dominant-negative calcineurin mutant or addition of cyclosporin, a calcineurin inhibitor, represses myotube differentiation and hypertrophy. Sustained exercise of a muscle also can activate

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calcineurin-mediated pathways leading to changes in the isoforms of myosin expressed and thus fiber type.

Recruitment of myogenic cells from adult pluripotent stem cells

While it has not been studied in the chicken, there is abundant evidence in other vertebrates, especially mammals, that there are cells within organs and tissues other than skeletal muscle that can contribute to the formation of muscle fibers following muscle injury[50]. These so call stem cells, have been well demonstrated in the bone marrow. Also stem cells exist within skeletal muscle which can reconstitute all the elements of the bone marrow following depletion of the endogenous hematopoeitic stem cells [51]. Stem cells for skeletal muscle repair can experimentally be demonstrated to exist in the circulation. The use of pluripotent stem cells able to move through the circulatory system has been evaluated as a means of repopulating regions of muscle damage produced by genetic disease [52]. What role such cells play in normal muscle development is not clear. Nor is it clear that stem cells have a role to play in strategies to increase meat production in birds. It is most consistent with the hypothesis that the function of these circulating stem cells is repair of damaged muscle, though in large measure this is the function of the adult myoblasts or satellite cells. Current evidence suggests that the stem cells found in skeletal muscle are not the satellite cell population [53]

Summary and Implications for Meat Production

It is widely recognized that the size and mass of muscle is controlled by three mechanisms, proliferation of muscle precursor cells, the number of muscle fibers formed, and hypertrophy of existent muscle fibers [25]. All mechanisms are operative throughout development, with the relative dominance of each differing during the three major phase of development - the embryonic, fetal, and neonatal/adult phases. During embryonic development proliferation and new fiber formation dominate, while during fetal development proliferation and new fiber formation continue but are more extensive than in the embryo, while hypertrophy begins. It is the fetal period during which the central nervous system through muscle fiber innervation comes to dominate. In the postnatal period hypertrophy alone dominates increases in muscle mass.
Muscle fiber types is determined by the lineage of myoblasts from which a fiber descended during its formation, functional innervation, and to some extent the degree to which the muscle is exercised. These factors determine the myosin genes that are

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activated and this in turn dictates the contractile function of a muscle.
All of these factors bear on meat production and all are potentially modifiable in a fashion that could lead to enhance meat production in the bird. One could reduce the inhibitory signals that restrain muscle formation or augment the stimulatory signals in the embryo. We have examples of this strategy in genetically modified cattle and in mice where there is marked increase in muscle mass or meat production by these procedures. Likewise we are increasingly understanding the pathways that control which myosin genes are expressed once a muscle fiber is formed [54]. These pathways have been genetically modified in mice with the result that patterns of muscle fibers have been altered. In principle it is feasible to increase ‚dark meat' formation and reduce ‚white' meat formation in the chicken by manipulating these mechanisms. The limitations in chicken of implementing genetic modifications that can enhance meat production have been in developing a technique to form transgenetic chickens. This procedure is under study in commercial laboratories and could lead to rapid changes in the meat production in poultry.

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RECENT ADVANCES IN TURKEY RYANODINE RECEPTORS

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Keywords: ryanodine receptor, malignant hyperthermia, PSE, alternative splicing, transcript variant

Abstract

We hypothesized that one or more mutation(s) in turkey ryanodine receptors (RYR) predispose birds to the development of PSE meat. Analysis of turkey *αRYR* cDNA covering amino acids 376 to 615 revealed three transcript variants. They were characterized by the presence or absence of 81 and 193 bp. The deletion of 81 bp results in the loss of 27 amino acids corresponding to Ser^{417}-Ser^{443}. The 193 bp deletion introduces an internal stop codon. Two *αRYR* DNA alleles corresponding to the deletions were identified. The two alleles were identical in exon sequences but the introns differed. Comparison of genomic and cDNA sequences suggests that the deletions arose via alternative splicing. Differences in turkey βRYR channel activity have also been identified

Résumé

Ce travail chez la Dinde repose sur l'hypothèse qu'une ou plusieurs mutations dans les récepteurs à la ryanodine pourrait accroître la sensibilité des animaux au défaut de type PSE. L'analyse des cDNA pour la partie du récepteur αRYR allant des acides aminés 376 à 615 a révélé trois formes de transcrits différentes, se distinguant par la présence ou l'absence de 81 ou 193 pb. La délétion de 81 pb se traduit par la perte de 27 acides aminés correspondant à Ser^{417}-Ser^{443}. La délétion de 193 pb conduit à l'introduction d'un codon stop. Deux allèles ont été identifiés au niveau de l'ADN génomique du récepteur αRYR, se différenciant uniquement au niveau d'un intron. La comparaison des séquences d'ADN complémentaire et génomique suggère que les délétions observées pourraient provenir d'un phénomène d'épissage alternatif. Des différences d'affinité du récepteur βRYR entre types génétiques ont également été rapportés.

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Introduction

A prominent problem found in turkey breast is meat which is characterized as pale, soft and exudative (PSE), a defect which appears to be very similar to the PSE pork problem identified in the 1960s (Ferket, 1995). Like PSE pork, PSE turkey exhibits unusually light color, and poor water holding capacity, protein extractability, and cook yield resulting from protein denaturation (Pietrzak et al., 1997).

The mechanism underlying the development of PSE meat is not clear, but environmental factors such as heat stress and pre-slaughter struggle clearly contribute to the magnitude of the problem (McCurdy et al., 1996; McKee and Sams, 1997). Genetics is also likely to play a contributing role in development of PSE meat by making birds more susceptible to stress. McCurdy et al. (1996) noted substantial variation in breast lightness among individual flocks of birds within a given season, suggesting a role for genetics in the stress response in turkeys, as was noted previously for swine. Stress-susceptible pigs are genetically predisposed to developing porcine stress syndrome (PSS) or malignant hyperthermia (MH), an inherited muscle disorder characterized severe muscle contracture coupled with excessive heat and lactic acid production, and augmented glycogenolysis and anaerobic glycolysis. An MH episode often results in death prior to slaughter (Mitchell and Heffron, 1982), whereas stress-susceptible pigs that go through the slaughter process often yield a high incidence of PSE meat (Lundström et al., 1989). However, it is important to note that normal and PSE meat can occur in both genetic lines, albeit with differences in frequency.

By the 1980's it was clear that an abnormal calcium release mechanism was a key factor underlying the development MH and PSE pork from stress susceptible pigs (Nelson, 1983; Cheah et al., 1984). The genetic basis for MH was identified in the early 1990's with the discovery of a point mutation in the skeletal muscle sarcoplasmic reticulum (SR) calcium-release channel, sometimes referred to as the ryanodine receptor (RYR) (Fujii et al., 1991). The mutation resulted in a change of arginine to cysteine at residue 615 of the RYR primary structure, which altered channel activity. A simple genetic test to test for the presence of the mutation has been widely used for screening of breeding stock (Zhang et al., 1992).

The genetic component underlying PSE turkey meat is still poorly understood. While MH is not a problem in turkeys, the striking similarity between PSE pork and turkey coupled with previously noted circumstantial evidence for a genetic component, make it likely that one or more mutations exist in turkey RY, which predisposes birds to the development of PSE meat. This article briefly reviews the role of RYRs in avian muscle contraction, the possible role of RYRs in development of PSE turkey, and presents some of our current work in this area.

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Ryanodine Receptors and Excitation-Contraction Coupling

The SR calcium release channel was first identified in SR vesicles of rabbit skeletal muscle as a ryanodine receptor protein (RYR1) because of its high affinity for ryanodine, a plant alkaloid originally investigated for possible insecticidal use. RYR1 is a homotetramer with subunit molecular mass of 565 kD, and each subunit consists of about 5000 amino acids (Takeshima et al., 1989, Zorzato et al., 1990). Analysis of the RYR1 primary structure reveals a short, cytoplasmic C-terminal domain and between four and ten membrane-spanning regions in the C-terminal one-fifth of the molecule. The transmembrane domains of each subunit monomer combine to form the pore of the channel. The N-terminal four-fifths of the molecule comprise the cytoplasmic domain, which can be observed by electron microscopy as a square-like « foot » structure. The foot structure extends across the gap of the triad junction from the SR terminal cisternae to the transverse tubule (T-tubule) membranes, where the dihydropyridine receptors (DHPR) are localized. During EC coupling, action potentials are conducted along the sarcolemma and into the interior of the muscle fiber via the T-tubule. The leading theory is that surface membrane voltage depolarization alters the conformation of the DHPR, which triggers release of calcium from the SR through the RYR1 via a physical DHPR-RYR1 link (Franzini-Armstrong and Protasi, 1997). Release of Ca^{2+} from the SR results in a rise in intracellular Ca^{2+} concentration, which activates the troponin complex, thereby initiating the contraction of the muscle.
Excitation-contraction (EC) coupling in avian skeletal muscle differs from that of mammalian muscle. Calcium release is effected in birds by two RYR isoforms (αRYR and βRYR) which are present in approximately equal abundance, rather than by one isoform (RYR1) in mammalian skeletal muscle (Airey et al., 1993). On the basis of sequence comparisons with the mammalian isoforms, the avian α and βRYRs are most similar in primary structure to the mammalian RYR1 and RYR3 isoforms, respectively (Ottini et al., 1996). O'Brien et al. (1995) proposed a two-component model for the calcium release mechanism in non-mammalian, vertebrate skeletal muscle. In this model, voltage-sensitive channels (αRYR) are gated via the DHPR in response to voltage changes across the T-tubular membrane, whereas the βRYRs likely operate by Ca^{2+}-induced Ca^{2+} release. Ca^{2+} released via αRYRs would activate neighboring "slave" channels. The β isoform is a logical candidate to be the calcium-coupled RYR isoform, because of its sequence and functional similarity to the cardiac (RYR2) and nonmuscle RYR's (RYR3), which operate by Ca^{2+}-induced Ca^{2+} release. Felder and Franzini-Armstrong (2002) have recently provided experimental support for this model. Their electron microscopy studies indicate that the β isoform is restricted to the parajunctional region of the triad and therefore, is likely activated via Ca^{2+}-induced Ca^{2+} release.

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Genetic Component of Malignant Hyperthermia and PSE Meat

MH is an autosomal dominant disorder of skeletal muscle Ca^{2+} regulation in humans and pigs. Thirty missense mutations and one amino acid deletion mutation in the primary structure of RYR1 have been associated with MH in human (Jurkat-Rott et al., 2000 and Sambuughin et al., 2001). Most MH-associated mutations in RYR1 cluster in two regions or "hot spots": hot spot 1 (residues 35-615) and hot spot 2 (residues 2162-2458). One mutation found in human MH is also found with porcine MH: $Arg^{615}Cys$. The $Arg^{615}Cys$ mutation of RYR1 causes the channel to be hypersensitive to stimulators of channel opening, such as μM [Ca^{2+}], and it does not close as readily in the presence of mM [Ca^{2+}] (Mickelson et al., 1988). When pigs carrying the homozygous mutant *ryr1* gene encounter stress before or during slaughter, their muscle cells are flooded with excess Ca^{2+}. This leads to sustained muscle contracture and attendant heat production, coupled with enhanced glycolytic and anaerobic metabolism. The combination of high pre-rigor temperature and low pH ultimately results in development of PSE meat. Based on the numerous mutations identified in human MH and their consequent effects on calcium regulation, we hypothesize that a mutation exists in either αRYR or βRYR or both, which predisposes turkey to the development of PSE meat.

Genetic Differences in Turkey *αRyR*

We screened turkey cDNAs for possible mutations of *αRYR* in the region corresponding to mutation hot spot 1 of human RYR1. We identified three different transcript variants covering nucleotides #1234-1757 corresponding to human RYR1 cDNA sequence. The longest transcript variant shared 70% amino acid sequence identity with mammalian RYR1 and bullfrog αRYR. Compared to turkey βRYR (our unpublished data), the amino acid sequence identity was 66%. The other two transcript variants were characterized by the absence of either 81-bp (AS-1; nucleotides #1350-1430) or 193-bp (AS-2; nucleotides #1350-1542). The 81-bp deletion would result in a 27-amino-acid deletion corresponding to Ser^{417}-Ser^{443} of human RYR1. The removal of 193-bp would lead to a frame-shift which would introduce a premature stop codon after amino acid residue #416. Thus, this transcript variant would not encode a complete functional channel protein.

Several different patterns of expression of *αRYR* transcript variants have been identified (Figure 1). We found some birds expressing only the transcript with no deletion (referred as wild type or W). There were also birds expressing combinations W, AS-1 and AS-2; W and AS-1; or AS-1 and AS-2. We have not yet identified any

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birds expressing either AS-1 or AS-2 alone. The patterns of expression suggest that either AS-1 or AS-2 or both are products of alternative splicing. The birds expressing AS-2 alone likely could not survive, because they would not have a functional channel protein.

The physiological significance of the 27-amino-acid deletion in the foot domain of turkey αRYR is still unknown. The deletion occurs in a region which is highly sensitive to amino acid changes as indicated by the clustering of eight mutations in this region in human MH (Jurkat-Rott et al., 2000). In addition, this region is within the cytoplasmic clamp domain which has been proposed to serve as part of the protein-protein contact site of RYR1 with the DHPR (Wu et al., 1997; Baker et al., 2002). Coupling of the DHPR and αRYR in avian skeletal muscle controls the opening and closing of αRYR during muscle contraction (O'Brien et al., 1995). Thus, a deletion in this region may alter the interaction and functional activity of these two proteins, which in turn could affect regulation of Ca-release.

Two *αRYR* Genomic DNA Alleles

The identification of the transcript variants prompted us to search for the origin of the deletions in the *αRYR* cDNA sequence. By analyzing the genomic DNA sequence in the region corresponding to W, AS-1 and AS-2, we identified two copies of *αRYR* alleles from different birds. We refer to these two copies of turkey *αRYR* genomic DNA as alleles *α–ryr-I* and *α–ryr-II*. Because the complete turkey *α–ryr* genomic DNA sequence is unknown, each intron and exon of both alleles was identified according to the corresponding intron and exon number of the human *ryr1* gene (Phillips et al., 1996). The sequences of both turkey genomic DNA alleles spanned from the last one third of exon 12, through intron 12, exon 13, and intron 13 to the end of exon 14.

The sequences of two alleles were compared. The exon sequences in alleles *α-ryr-I* and *α–ryr-II* were identical to each other and to the turkey cDNA sequence. However, the two alleles were different in their introns. The difference of introns was demonstrated by their different restriction enzyme digestion pattern (Figure 2). The size of intron 12 in *α–ryr-II* is approximately 1 kb longer than that in *α–ryr-I*. The significance of the difference in intron 12 between the alleles is not clear at this point.

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Genotypes of Turkey and the Corresponding Expression of *αRYR* Transcript Variants

Turkeys could be grouped into three different genotypes based on the two α-*ryr* alleles: homozygous *α-ryr-I*, homozygous *α-ryr-II*, and heterozygous *αryr-I* and *α-ryr-II* turkeys. Compared to the genomic DNA sequence, the three *α-ryr* transcript variants were characterized as:

1) W transcript variant, which has sequences of exons 12-14 identical to genomic DNA exon;
2) AS-1 transcript variant, which has a deletion of 81 bp located at the beginning of exon 13;
3) AS-2 transcript variant, which has a deletion of 193 bp, corresponding to the entire exon 13.

There was no evidence that the missing nucleotides in the cDNA sequence was the result of deletion mutations in the genomic DNA sequence. Therefore, we suggest that the different transcript variants came from the result of alternative splicing. Consistent with this hypothesis was the observation that the last three nucleotides of the 81 bp deletion (CAG) form a consensus sequence for a splice acceptor site. As we screened genotypes of turkey and the corresponding expression of the mRNA transcript variants, we concluded that birds expressing the transcripts with either 81-bp or 193-bp deletions were homozygous for *α-ryr-I* or *α-ryr-II* or were heterozygous. This suggests that alternative splicing occurred in both *α-ryr* alleles. However, splicing frequency of the two alleles still needs to be investigated.

Evidence for Functional Differences in Turkey βRYR

The activity of RYR is often measured by the affinity of the receptor to its ligand, ryanodine. Increased affinity of a RYR for ryanodine compared to a wild-type RYR is consistent with a higher probability of the channel being open under a given set of conditions. Altered affinity of RYR for ryanodine is also consistent with a change in primary structure. We modified the ryanodine binding assay of Mickelson et al. (1988) to screen turkey skeletal muscle SR preparations for altered RYR activity. We examined a fast-growing commercial line, and a random-bred, genetically unimproved line of turkeys. Our results were consistent with those observed for stress-susceptible and normal pigs. One commercial turkey group had a significantly higher affinity for ryanodine compared to our genetically unimproved turkeys. We also found another commercial turkey group had approximately the same affinity as the random-bred turkey group (Wang et al., 1999; Zhang, 2000). These differences suggest that there is

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heterogeneity of the RYR channel activity between the random-bred and commercial turkey populations.

Until recently, it was unclear whether this difference in ryanodine-binding activity represents differences in the αRYR or in the βRYR, or in both isoforms. A recent study of bullfrog RYR channel activity in SR vesicles may prove informative. Murayama and Ogawa (2001) demonstrated that in frog SR vesicles, which are structurally and functionally similar to turkey SR, the αRYR ryanodine binding activity is almost completely suppressed. The ryanodine binding observed in frog SR is the result of βRYR activity. If confirmed in turkey SR, our results would suggest that the differences RYR channel activities in our study could be ascribed to differences in amino acid sequence in the βRYR isoform.

Conclusions

Mutations in RYR1 have been highly correlated with human and porcine MH, and a point mutation in pig RYR1 is underlying the PSE pork development. The similarity of the development of PSE turkey and PSE pork suggests that RYR could be a possible genetic component to the PSE problem in the turkey industry. In this article, we provide some evidence of the differences in both turkey αRYR and βRYR. We expect that the genetic differences of αRYR and channel activity differences in βRYR will affect the functionality of each RYR isoform. The correlation of the differences with meat quality traits will be significant in understanding molecular basis underlying the PSE turkey problem.

Figure 1. Analysis of turkey *αRYR* transcript variants corresponding to nucleotides #1234-1757. The identification numer of each bird is shown on the top of the gel. L100 and L50 are 100-bp and 50-bp DNA ladders, respectively. The wild type variant is indicated by *, the AS-1 is indicated by the arrowhead, and the AS-2 is indicated by the arrow.

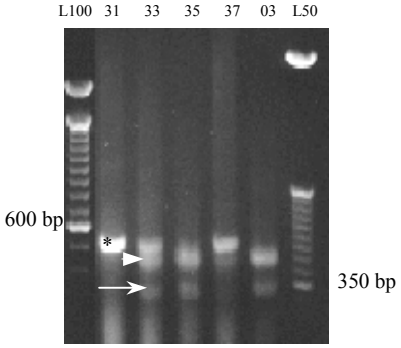

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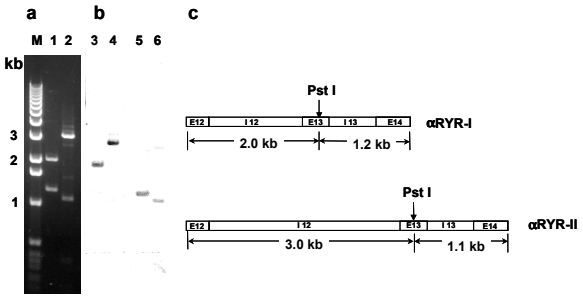
Figure 2. Partial structure of turkey *αRYR* alleles derived from restriction digestion by *PstI*. a) The digested fragments of *αRYR-I* (lane 1) and *αRYR-II* (lane 2) were analyzed by an agarose gel electrophoresis. b) The digested fragments were transferred to nylon membrane and probed either with E12 (lanes 3 and 4) or E14 probes (lanes 5 and 6) in the Southern hybridization analysis. c) Reconstruction of the fragments of each allele. M: 1kb DNA ladder



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EARLY POST-MORTEM pH AND TURKEY BREAST MEAT QUALITY

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Keywords : turkey, meat quality, pH drop, two dimensional electrophoresis, muscular proteins

Abstract

In this study, the properties of fast and normal glycolyzing turkey breast muscles concerning their meat quality and protein alterations are studied. In a commercial processing plant, in a large flock, seventeen breast muscles were selected with a low pH at 20 min post-mortem (fast glycolyzing, FG; pH_{20min} = 6.04 ± 0.09) and seventeen with a normal pH_{20min} (normal glycolyzing, NG; pH_{20min} = 6.47 ± 0.01). Several measurements were done on both groups. No differences in L* values were measured during a 9-day storage period at 4°C between the 2 groups. The FG group showed a lower water holding capacity and lower processing yield compared to the NG group. Warner-Bratzler shear force values of cooked meat were higher for the FG group than for the NG group. The FG meat presented a lower protein extractability than the NG one with a low ionic strength (LIS) buffer. However, no differences between the 2 groups were reported in protein extractability with high ionic strength buffer. LIS protein extract is also studied using 2 dimensional electrophoresis and gels are shown.

Résumé

Les propriétés technologiques et certains facteurs biochimiques de viande issue de dindes à glycolyse rapide et lente seront étudiés. Dans un abattoir, au sein d'un même lot, 17 muscles pectoraux ont été sélectionnés avec de basses valeurs de pH à 20 min post-mortem (glycolyse rapide, GR, pH_{20min} = 6.04 ± 0.09) et 17 avec des valeurs normales de pH (glycolyse normale, GN, pH_{20min} = 6.47 ± 0.01). Les valeurs de L* ne diffèrent pas entre les 2 groupes au cours de la conservation à +4°C pendant 9 jours. Le groupe GR présente une plus faible capacité de rétention en eau et un moindre rendement Napole. La force de cisaillement de la viande cuite est plus élevée pour le groupe GR que pour le groupe GN. La viande du groupe GR présente une plus faible extractabilité des protéines à faible force ionique (FFI). Cependant, l'extractabilité des

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protéines à force ionique élevée ne diffère pas entre les 2 groupes. La fraction FFI est également étudiée en électrophorèse bidimensionnelle et des gels sont présentés.

Introduction

The pale, soft, exudative (PSE) syndrome leads to meats, which have a paler colour, a higher toughness and a lower water-holding capacity. In pork, this phenomenon has been described a long time ago while it has been reported more recently in poultry. However, in all cases PSE meat appears when a fast pH fall occurs early post-mortem. This fast glycolysis leads to a low muscular pH when the temperature of the muscle is still high. The combination of these 2 parameters may lead to the denaturation of muscular proteins. The loss of protein functionality is often suggested to be responsible for the alterations in meat quality. In turkey, some studies reported that birds with a high rate of pH fall had a lower meat quality (Pietrzak et al., 1997 and Rathgeber et al., 1999a). The aim of the work is to study the technological properties and some biochemical factors of turkey breast meat showing different rate of pH fall.

Material and Methods

In a commercial processing plant, pH of 430 BUT9 tom turkeys was measured at 20 min *post-mortem* (Jeacocke, 1977). From this, 2 groups of animals were done : the first one (Normal Glycolysis, NG, n = 17) was made up of animals with a "normal" pH_{20min} (6.47 ± 0.01) and the second one (Fast Glycolysis, FG, n = 17) of birds with the lowest pH_{20min} (6.04 ± 0.09). The ultimate pH was 5.67 in the 2 groups. At 24h post-mortem (D1), scallops were harvested for different measurements during a 9-day storage period at 4°C.

The colour of the meat was measured at D1, D3, D6 and D9 on the scallops with a Minolta CR-300.

Drip loss was evaluated at D3, D6 and D9 and is expressed in percent of the weight at D1. Thaw loss was also evaluated after a10-day storage period at –20°C. Cook loss was measured according to Honikel (1998).

Napole yield was determined using the procedure described by Naveau et al. (1985).

Objective texture was evaluated according to Honikel, 1998.

Buffering capacity was evaluated according to Monin and Sellier (1985).

Protein extractabilities were measured using the modified procedures described by Rathgeber et al. (1999a) and Pietrzak et al. (1997). Protein amounts in low ionic strength (LIS) extracts, high ionic strength (HIS) extracts and residual proteins

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remaining after extraction by HIS (pellet) were determined using BCA Protein Assay Kit (Pierce, Rockford, IL, USA). Results are expressed as a percentage of total proteins.
Two-dimension gels of the LIS extract were performed on immobilized pH gradient (IPG, 7 cm) strips of 7-10 according to Görg et al. (2000).
Data were analyzed by analysis of variance using the general linear model procedure of Minitab® software (1994).

Results and discussion

Whatever the time considered, L* values never differ between the 2 groups. Pietrzak et al. (1997) reported higher L* values for PSE turkey breast meat ($pH_{20min} < 5.8$) than for normal meat ($pH_{20min} > 5.8$). However, Rathgeber et al. (1999) did not report such a result for PSE and normal turkey meat. From the present study, L* value of meat is not modified by a higher rate of pH fall.
Drip loss, thawing and cook losses were higher for FG meat than for NG one (**Figure 1**). Few studies reported the influence of rate of pH fall on drip loss during a 9-d storage period but Mc Kee and Sams (1998) also reported higher cook loss values for PSE turkey meat. Napole yield is an indicator of brine and cook yield. It is similar to conditions encountered in processing plants. FG meat had a lower Napole yield than NG one (99.2 ± 1.4 et 101.3 ± 1.7% respectively). Pietrzak et al. (1997) and Rathgeber et al. (1999a) also reported lower processing ability of PSE breast meat compared to normal meat. We conclude that a high rate of pH fall induced a lower water holding capacity of turkey meat.
FG meat had a higher maximum shear force value than NG one, markedly when the meat is cooked (29.4 ± 5.8 and 20.9 ± 3.5 N, respectively). As in our study, Mc Kee and Sams (1998) reported higher shear force value for cooked meat in PSE turkey. We conclude that tenderness of PSE meat turkey was lower than normal meat.
Buffering capacity of FG meat was lower than for NG meat (48.8 ± 2.4 and 51.4 ± 2.0 meq H^+/(pH x kg) respectively, $P < 0.01$). Monin and Sellier (1985) reported no differences in buffering capacity of muscles from various pork genotypes leading or not to PSE syndrome development. In our study, lower buffering capacity of FG group may be linked with a loss in protein functionality.

LIS protein extractability was lower for FG meat than for NG meat (24.6 ± 1.1 and 25.4 ± 0.9% respectively). On the contrary, no differences were found between the 2 groups for HIS protein extractability. Rathgeber et al. (1999a) reported a lower extractability for sarcoplasmic (similar to our LIS extract) proteins, but also for and

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myofibrillar (similar to our HIS extract) proteins. Nevertheless, we conclude that a fast rate of pH fall led to lower protein extractabilities.
In order to make a link between buffering capacity, protein extractability and the technological properties, we investigate protein extracts using 2 dimensional electrophoresis. **Figure 2** shows 2 dimensional gels of LIS extracts from NG and FG animals. The 2 groups clearly show different patterns. At least 4 spots located between pHi 8 and 9 and molecular weight around 40-60 kDa can be seen in the NG group whereas it is not in the FG group. Another spot (pHi around 9 ; molecular weight around 15-20 kDa) can be seen in the FG group only.
In this study, 2 dimensional gels clearly show that there are alterations of LIS extract proteins. We have now to better identify these differences and make the link between the observed alterations of meat quality such as water holding capacity, tenderness, processing yield…
The authors would like to thank the Groupe Coopératif Occitan (Castelnaudary, France) and Linea Alimentaria (Serinya, Spain) for providing turkey breast muscle.

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Figure 1. Drip loss during a 9-d storage period, thaw loss and cook loss in FG and NG turkey breast meat (mean ± SD, n = 17). * P < 0.05; ** P< 0.01 et *** P< 0.001

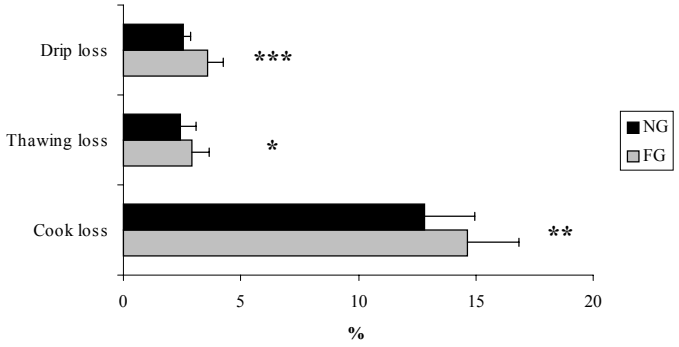
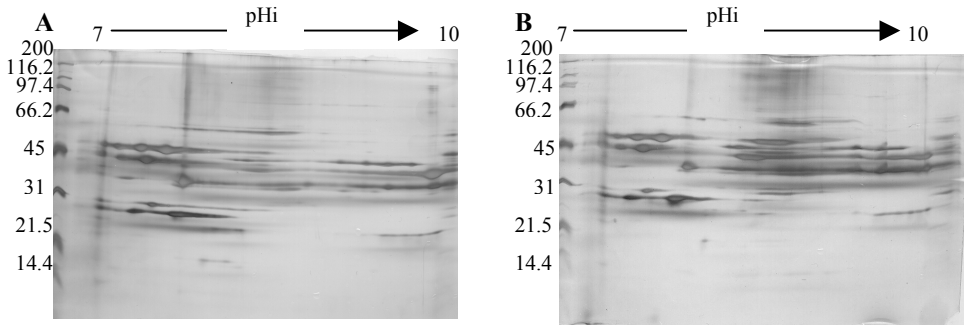


Figure 2. Two-dimensional gels of LIS extracts from FG (**A**) and NG (**B**) animals. First dimension, IPG 7-10, separation distance 7 cm; Second dimension SDS-PAGE (15%T constant).



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CHARACTERIZATION OF TURKEY BREAST MEAT QUALITY USING COLOUR IMAGE ANALYSIS

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Abstract

In the past decade, the turkey meat industry has developed new products based on cured and cooked meat. Processors need to adapt the meat quality to further processing. The technological quality of the processed meat depends on water holding capacity of fresh meat, which is correlated with pH values. A previous study has shown that early pH determines partly the colour of the fresh meat (Santé *et al*., 1996). The aim of the present study is to use on line colour video image to predict turkey breast meat yield and consumer attitude toward fresh meat. The results showed that the lightness L* is correlated with cured and cooked breast yield : the higher the lightness the lower the yield. In our experimental conditions, for L* values above 55, yield was less than 90%. Moreover, consumer study revealed that the lighter colour of breast meat was preferred to the darker one. In conclusion, the lighter meat could be moved to fresh meat and the darker one for processing.

Résumé

Au niveau industriel, la prise en compte de la qualité technologique est devenue de plus en plus importante avec le développement de produits élaborés à base de viande de dinde. Pour les produits transformés par saumurage et cuisson, le rendement dépend de la capacité de rétention d'eau de la viande. La présente étude a pour objectif d'évaluer l'intérêt de la vision numérique comme instrument de tri en ligne des filets de dinde destinés à la transformation par saumurage et cuisson. Les résultats montrent que les groupes de filets présentant les indices de luminosité les plus élevés (L* vision, filets plus clairs) sont aussi ceux qui ont le rendement à la cuisson significativement le plus faible. Les tests d'acceptabilité par le consommateur montrent que ceux-ci préfèrent les viandes plus pâles.

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Introduction

The technological quality of breast meat is an increasingly critical factor for processed turkey meat products. The cooking yield is a major quality trait for processed products such as cooked turkey breast ham. Cooking yield is closely dependent upon the water holding capacity (WHC) of the meat. Colour is correlated to WHC and cooking yield in poultry meat. Its measurement by image analysis is fast, non-invasive and compatible with the production rate in meat processing plants. The aim of the study is to use video image colour on line to sort meat by its colour, determine its meat yield and evaluate the consumer attitude toward 4 colour classes of fresh meat

Material and methods

A total of one hundred and seventy turkey breast muscles were collected in a slaughter plant from 4 trials. The breast meat was obtained from 15-week old male turkeys (line BUT 9) after conventional chilling over a minimum of 8 hours. Firstly selected visually to obtain the largest range of colour, from lightest to darkest, the breast meat colour was measured using a tri-CCD DXC 990 P SONY camera and analysed according to the method developed by CEMAGREF (Marty-Mahé *et al*., 2002). The ultimate pH value (pHu) was determined 24 h post mortem using a portable pH meter equipped with a glass electrode directly inserted into the muscle. The results were expressed as L*, a*, b*, C* and h* coordinates for lightness, redness, yellowness, chroma ($C= (a^{*2}+b^{*2})^{1/2}$) and hue angle (h=arc tang (b*/a*)), respectively. The breast meat was subsequently vacuum packaged, immediately frozen and stored at –20°C for further analysis. After thawing, the cooking yield was measured on a muscle sample of standard size (15 x 5 x 2,5 cm; 337 ± 14 g, n=170) after injection of brine (136 g of nitrite salt x l^{-1}) at the rate of 15 %, vacuum packaging and cooking in a water bath up to a 68°C internal temperature was reached. Consumer tests were repeated 4 times on fresh meat. A total of 320 persons were polled with respect to class of age, men/women ratio etc…
The meat quality data were subjected to analysis of variance using the general linear model procedure of SAS, and the means were separated using the Scheffe's test. A non-parametric test (Friedman) was performed for consumer data, means were compared by Wilcoxon test.

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Results

The muscle samples were distributed in 4 groups according to their L* value obtained by image analysis: 45-48 (class 1), 49-52 (class 2), 53-56 (class 3), and 57- 60 (class 4). The proportion of the total number of muscles studied for each class was 10, 39, 33 and 18%, respectively. As expected, the average cooking yield decreased as the L* value increased (or the muscles were lighter; Table 1). Significant differences between mean pHu values according to the treatment were observed, with the exception of classes 1 and 2.
Lightness showed a bimodal distribution (Figure 1). The peaks were observed at L* values of 51 and 56, and we may assume that they correspond to populations of muscles of normal and deficient quality, respectively. Similarly, the distribution of cooking yields showed two populations with a transition at around 91% yield (Figure 2). When L* is above 55, there is a greater number of muscles with cooking yield ≥ 90% than those with cooking yield < 90%.

Table 1. Colour, pHu and cooking yield according to lightness (L* value) as measured by image analysis

	Class of lightness (limit values of L* by image analysis)				p
	1 (45-48)	2 (49-52)	3 (53-56)	4 (57-60)	
No. of muscles	18	66	56	30	
Image analysis:					
L*	47,7 [a]	51,3 [b]	55,3 [c]	58,2 [d]	***
a *	19,5 [a]	19,4 [a]	17,7 [b]	17,4 [b]	***
b*	9,2 [a]	9,8 [a]	10,1 [a]	11,0 [b]	***
C *	21,6 [a b]	21,8 [a]	20,5 [b]	20,7 [b]	***
h *	0,44 [a]	0,47 [a]	0,52 [b]	0,57 [c]	***
pHu	5,95 [a]	5,94 [a]	5,85 [b]	5,79 [c]	***
Cooking yield (%)	93,7 [a]	92,6 [a]	90,5 [b]	89,2 [b]	***

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Fig 1. Distribution of lightness values (L*, image analysis)

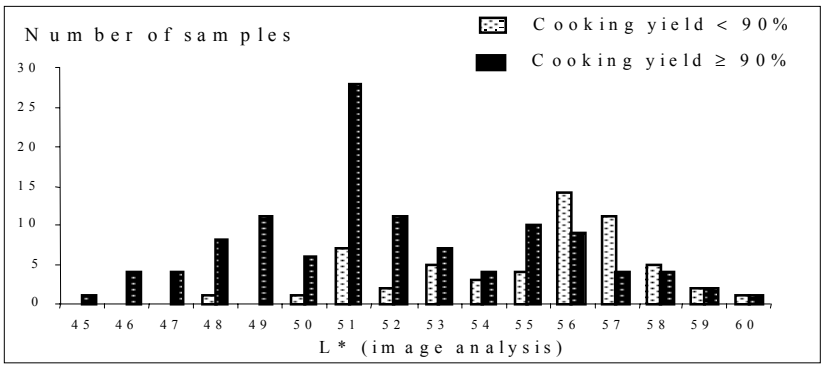

Fig 2. Distribution of cooking yield

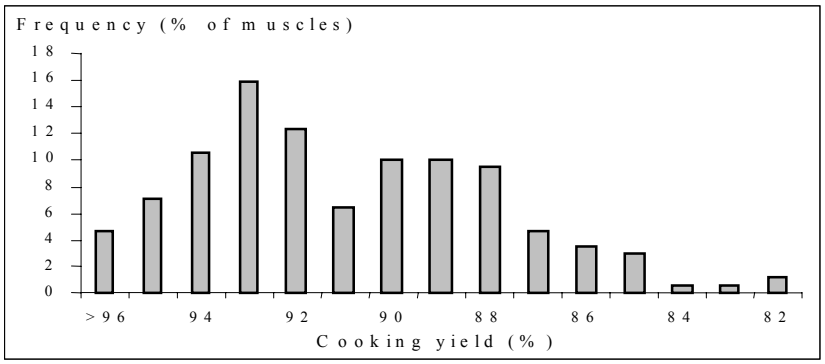

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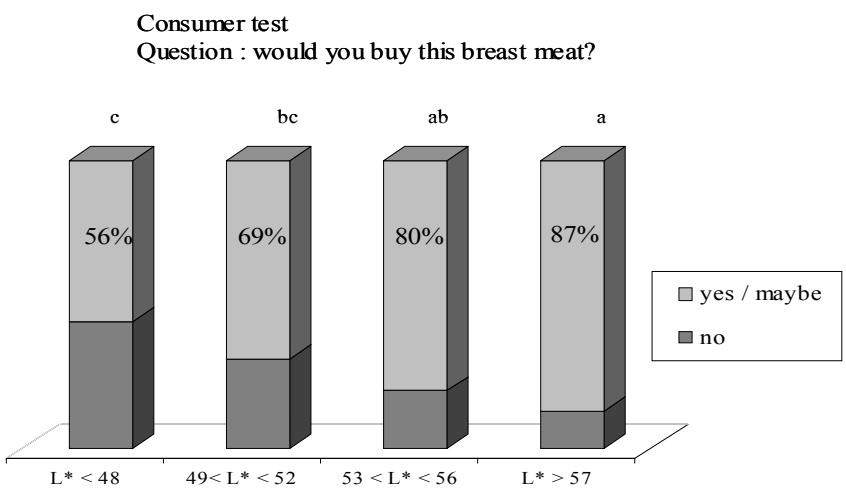
Table 2. Selection of breast muscles on the basis of L* values: percentage of selected muscles and corresponding average cooking yield (CY).

L* limit value	% muscles		Average CY (%)
	Selected	CY ≥ 90%	
<48	8,8	100,0	93,7
48	10,6	94,4	93,7
49	17,1	96,6	93,3
50	21,2	94,4	93,3
51	41,8	87,3	92,2
52	49,4	86,9	92,9
53	50,6	81,4	91,2
54	60,6	81,6	91,1
55	68,8	80,3	91,5
56	82,4	73,6	89,4
57	91,2	69,0	89,4
> 57	100,0	67,3	89,1

The result of a selection of muscles on the basis of an L* value greater than a limit value is presented in Table 2. The cooking yield showed a marked decrease with the increase in the L* value above 55. If this value was chosen as a threshold, 69% of the breast muscles would be selected, with an average cooking yield of 91.5%

Figure 3 : consumer acceptability test



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Consumer tests were performed to evaluate acceptability of fresh meat and determine what they like/dislike in the 4 different classes of meat colour. The lighter meat (L*>53) has got the best result in term of acceptability, mainly because consumers like its colour. On the other hand, the darker class was disliked because of its colour. Besides the colour, no trait of the general aspect of the meat was outstanding (fibre, freshness etc…). When asked about their intention of buying the product, the lighter colour class collected the best results (Fig 3).

Conclusions

The results of this study showed that a relationship exists between the cooking yield of brine injected turkey breast muscles without water binding additive and the colour coordinates measured by image analysis. The measure of the colour on line could be performed to sort the meat according to its processing quality. The darker meat gave the best results for processing and consumers prefered the lighter meat.

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Acknowledgements

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BIOCHEMICAL DETERMINISM OF ULTIMATE pH IN BREAST MUSCLE OF BROILER CHICKEN

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Keywords : pH_u, glycogen, phosphoric components, buffering capacity, AMPd.

Abstract

The objective of this experiment was to study the biochemical determinism of ultimate pH (pH_u) in the *Pectoralis* muscle of broiler chickens. Thirty birds of each of 3 genetic types (a Standard, a French Label and a Heavy line) were slaughtered over the same day. The pH_u differed significantly among the 3 genetic types, ranking as: Standard > Heavy > Label. In Label and Heavy birds, ultimate pH was strongly correlated with the content of glycogen at slaughter, whereas this correlation was very poor in standard birds. Regardless of genetic type, neither buffering capacity nor lactate accumulation significantly contributed to pH_u variations. The activity of AMP deaminase was significantly higher in Standard birds than in the two other types. Further research is needed to study in more details the role of this enzyme in the determinism of pH_u.

Résumé

L'objectif de cette expérience était d'étudier le déterminisme biochimique du pH ultime (pH_u), dans le muscle pectoral de poulets. Quatre vingt dix poulets de 3 types génétiques (Standard, Label et Lourd) ont été abattus simultanément. Le type génétique affecte significativement le pH_u, selon le classement : Standard > Lourd > Label. Pour les souches Label et Lourd, le pH_u dépend essentiellement de la quantité de glycogène à l'abattage. Toutefois cette corrélation est faible chez les poulets Standards. Quel que soit le type génétique, ni le pouvoir tampon, ni l'accumulation d'acide lactique ne contribuent significativement aux variations de pH_u. L'activité de l'enzyme AMPd est significativement plus élevée chez les poulets Standard que chez

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les deux autres types génétiques. Le rôle de cet enzyme dans le déterminisme du pH_u mérite d'être étudié plus en détail.

Introduction

Meat quality is influenced to a large extent by the ultimate pH (Sales & Mellett, 1996). Muscle glycogen is the main fuel for the anaerobic glycolysis when muscles are nolonger supplied with oxygen. Thus, in mammals, the ultimate pH of meat chiefly depends on the muscle glycogen content at time of slaughter (Bendall, 1973). However, the pH fall can be stopped even in the presence of high residual glycogen content (Bendall, 1973). According to Scopes (1971), the progressive deamination of AMP to IMP, and the subsequent inactivation of glycolytic enzymes resulting from AMP disappearance, could explain the stop of pH fall even in the presence of glycogen.
By contrast, the determinism of ultimate pH has been little studied in poultry species. We recently recorded in turkey *Pectoralis major* muscle a lack of relationship between glycogen content at slaughter and ultimate pH (Fernandez, unpublished observations). This would suggest that the biochemical determinism of ultimate pH in poultry species differs from that in mammals. Therefore, the present experiment was carried out as a first step into the study of the determinism of pH_u in broiler chicken.

Materials and Methods

Thirty birds from each of three genetic types (Standard, French Label and Heavy line) were kept under conventional breeding methods at the Poultry Research Centre (INRA- Nouzilly) until the usual marketing age (6, 12 and 6 weeks for standard, label and heavy birds, respectively). They were slaughtered on the same day at the experimental slaughterhouse of the INRA Poultry Research Centre. Before slaughter, they were divided into 3 groups differing in the *ante mortem* treatments: minimum stress, shackling for a longer time (2 minutes) and "heat stress" (exposition to 35°C during 3.5 h and shackling for 2 min before stunning). The birds were electrically stunned in a water-bath and killed by a ventral neck cut.
At 3 min *post mortem* (*p.m.),* the pH was measured in *Pectoralis major* (PM) muscle as described by Jeacocke (1977). At 24 hours *p.m*., the pH was measured by direct insertion of a combined electrode in the muscle. Buffering capacity of PM muscle was measured at 3 min *p.m*. as described by (Monin & Sellier, 1985). Glycogen, glucose, glucose-6-phosphate and lactate were measured by enzymatic procedures at 3 min and

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24 hours *p.m*. according to Dalrymphe & Hamm (1973). Glycolytic Potential (GP) was calculated according to Monin & Sellier (1985) as follows:
GP = 2 ([glycogen] + [glucose] + [glucose-6-P]) + [lactate]
expressed as µmol lactate equivalent / g fresh tissue.
GP takes into account the main intermediates of glycogen degradation in live and *post mortem* muscle (Monin & Sellier, 1985) and represents therefore an estimation of resting glycogen level.
"High energy" phosphate compounds (ATP, AMP, and IMP), creatine phosphate and creatine were measured at 3min and 24 hours *p.m*., according to Monin *et al*. (1995). AMP deaminase (AMPd) activity was measured by HPLC at 3 min *p.m.* as described by Norman *et al*. (1994).
The data were statistically evaluated using the SAS system. Variance analysis was carried out using the GLM procedure of SAS. The model included the effect of genetic type, pre-slaughter treatment and their interaction. In addition, Spearman correlation coefficients were calculated.

Results and discussion

There was no significant effect of pre-slaughter treatment on any trait under study. In addition, the interaction between genetic type and pre-slaughter treatment was never significant. Therefore, in the following, the results will be presented according to genetic type only. The ultimate pH differed significantly among the three genetic types (figure 1). The Standard line showed the highest pH_u, the Label the lowest, whereas the Heavy Line showed an intermediate value.

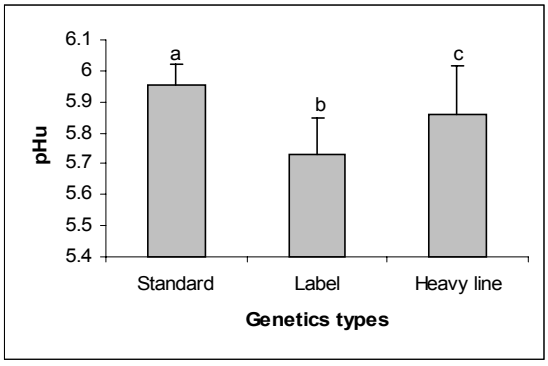

Figure 1: Values of pHu in the 3 genetic types; abc: Different letters indicate significant differences ($p<0.05$)

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The correlations between pH_u and biochemical data within the 3 genetic types are presented in table1.
The pH_u is highly correlated to glycogen level and glycolytic potential at 3 min *p.m*. in the French Label and Heavy line types. However in the Standard type, these relations are not statistically significant. These relationships are illustrated on Figure 2.

Table1: Correlations between pH_u and biochemical measurements (**, $p < 0.01$)

	Standard	**Label**	**Heavy Line**
3 min *post mortem*			
Glycogen	-0.30	-0.61 **	-0.76 **
Glycolytic potential	-0.34	-0.74 **	-0.82 **
Creatine	-0.07	-0.43 **	0.08
Creatine phosphate	-0.06	-0.29	-0.01
IMP	0.1	0.04	0.08
AMP	0.13	-0.04	0.14
ATP	0.17	-0.11	0.42 **
Buffering capacity	0.32	0.03	0.20
24 h *post mortem*			
Glycogen	-0.19	-0.59 **	-0.63 **
Lactate	-0.05	-0.28	-0.29
Creatine	0.04	-0.31	0.09
Creatine phosphate	-0.01	-0.15	0.06
IMP	0.13	-0.32	0.15
AMP	-0.03	0.24	-0.08
ATP	0.17	-0.11	0.42 **

In Standard chickens, as well as in the other genetic types, variations in pH_u are not explained by the accumulation of lactate during rigor process or by buffering capacity (see table 1). While studying PSE characteristics in broiler breast meat, Van Laack (2000) reports similar result, since this author does not find significant correlation between lactate at 24 hours *p.m*. and pH_u.
The present results show that i) the variations in pH_u of Standard chickens cannot be explained by usual biochemical parameters such as glycogen level and/or buffering capacity, and ii) for similar glycogen concentration (see figure 2 for values above 100 µmol/g) the pH_u is higher in Standard birds than in the other types. According to Scopes (1971), when glycogen is not a limiting factor, pH_u could be determined by AMPd activity since the disappearance of AMP induces an inhibition of biochemical reactions in *post mortem* muscle. As shown in Figure 3, the activity of AMPd is significantly higher in the *Pectoralis major* of Standard chickens than in Label and Heavy birds. This result is consistent with the fact that ultimate pH is higher in

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Standard birds than in the two other types, with similar glycogen concentrations at time of slaughter.

Conclusion

The present results demonstrate genetic differences in the determinism of pH_u in broiler chicken. Differences in mean pH_u values between genetic types could be attributed to differences in the activity of AMP deaminase. The implication of this enzyme in the determinism of pH_u in poultry muscle deserves further investigation.

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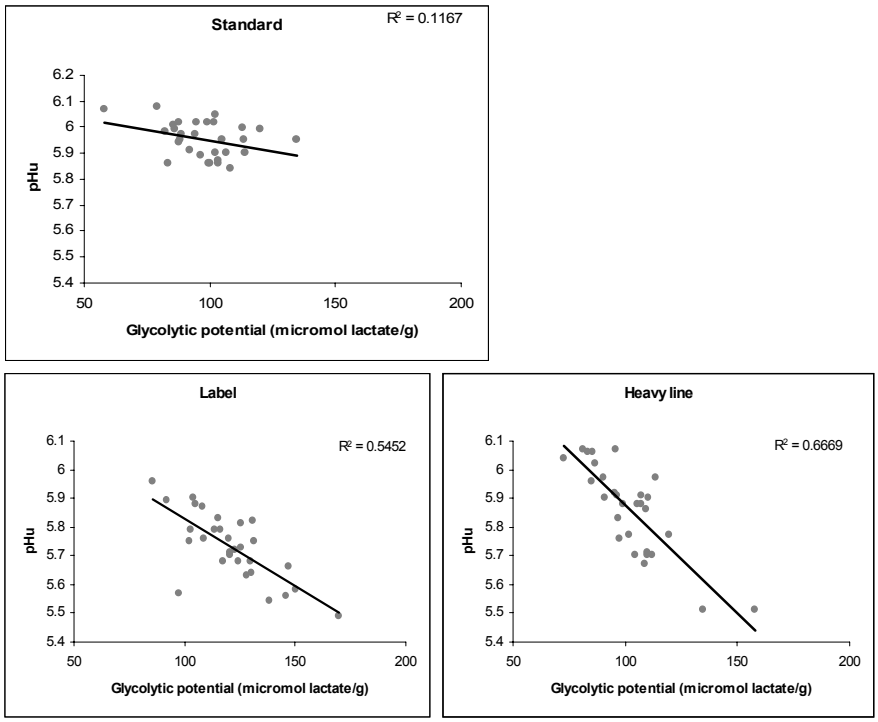

Figure2: Relationships between pH_u and glycolytic potential at 3 min *p.m.*

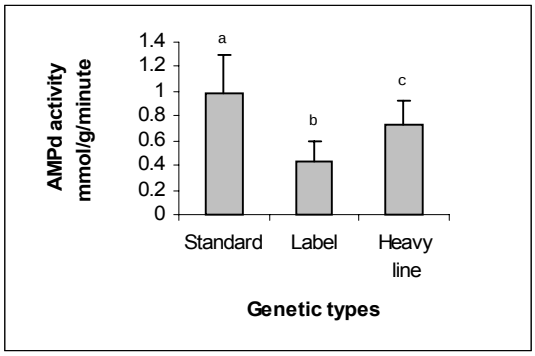

Figure3: AMPd activity in the 3 genetic types
abc: Different letters indicate significant differences ($p<0.05$)

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CHARACTERISTICS OF PALE AND DARK BROILER BREAST MEAT

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Keywords : broiler, breast meat, colour, pH, water holding capacity.

Abstract

A study was conducted to determine the functional properties of pale and dark broiler breast meat. A total of 216 breast fillets were collected from the deboning line of a commercial processing plant. Breast fillets were selected based on lightness values as follows: dark (L*<50; n=72), normal (50<L*<56; n=72;) and pale (L*>56; n=72) fillets. The breast fillets were kept intact or ground and used to determine ultimate pH, drip loss, cooking loss, moisture after cooking, shear value and LR-NMR parameters. There were significant differences (P<0.01) among the L*-groups in pH and WHC (cooking loss and NMR parameters). These results confirm that pale breast meat is associated with a lower pH values and poorer WHC measured by both standard methods and LR-NMR technique.

Résumé

L'étude a été réalisée pour évaluer les propriétés fonctionnelles du filet de poulet de chair en relation avec sa luminosité (L*). Au total, 216 filets ont été prélevés sur un site industriel de transformation et répartis en 3 lots: sombre (L*<50; n=72), normale (50<L*<56; n=72) et pâle (L*>56; n=72). Les filets entiers ou hachés ont été analysés pour le pH ultime, les pertes de jus par exsudation et à la cuisson, la teneur en eau après la cuisson, la force de cisaillement et les paramètres RMN-basse résolution. Des différences significatives (P<0,01) entre les lots ont été observées pour le pH ultime et le pouvoir de rétention d'eau (PRE) (pertes à la cuisson et paramètres RMN). Ces résultats confirment que la viande de couleur plus claire présente des valeurs de pH plus bas et à un PRE plus faible mesuré par des méthodes standard ou par la technique RMN.

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Introduction

In recent years, there has been concern regarding the fact that commercial chickens may exhibit meat quality problems ranging from dark, firm and dry (DFD) to pale, soft and exudative (PSE)-like characteristics. Both DFD-like and PSE-like meat have been associated with poor functionality properties, or at least, have been considered major contributing factors to product variations (Fletcher, 2002). Considerable attention has been focused on the PSE-like condition in poultry meat because of major quality problems in further processed and cooked produce. The PSE-like poultry meat causes its greatest problem in products to which no or low amount of salt and phosphates are added to enhance the water holding capacity (WHC) reducing cooking and drip losses. The opposite set of conditions occurs in the case of DFD-like meat where the WHC is greatly improved. This high WHC is beneficial for further processing as the added moisture and ingredients are more tightly held within the meat, however, the dark appearance of fresh poultry is considered unacceptable by consumers (Mallia *et al*., 2000). A number of scientific papers has recently suggested the possibility of using objective colour measurements to predict functional properties of poultry (Barbut, 1997; Wilkins *et al*., 2000; Woelfel *et al*., 2002). Low-Resolution Nuclear Magnetic Resonance (LR-NMR) has been proposed as an alternative technique to investigate WHC in pork (Brown *et al*., 2000; Bertram *et al*., 2001) and poultry (Cremonini *et al*., 2001; Cavani *et al*., 2002). The study of NMR relaxation properties can give relevant information about compartmentalisation, dynamics of water as well as the microstructure and WHC of the meat through the measure of transverse relaxation time (T_2). The aim of this study was to assess the colour variation of broiler breast meat in a commercial processing plant as well as the quality characteristics of pale and dark breast meat. Specific goal included an evaluation of the powerful of LR-NMR technique to discriminate broiler breast meat functional properties.

Material and Methods

A total of 6997 broiler breast fillets (*P. major* muscles) from 54 flocks were assessed for colour throughout an 8 month period (June 2002-February 2003) in a single major italian processing plant. Whole breast fillets were randomly collected from the deboning line at 6 h *post mortem*. Colour measurements were performed using a Minolta Chromameter CR-300 with illuminant sources C and colour was expressed in terms of CIELab values for lightness (L*), redness (a*) and yellowness (b*). Colour was measured on the cranial, medial surface (bone side) in areas free of colour defects (bruises, discolorations or any other condition that might have affected uniform colour

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reading). Furthermore, a total of 108 boneless, skinless, 24 h *post mortem* whole breast muscles (216 breast fillets) from 8 wk-old male broilers (3.5 kg live wt) were selected based on lightness (L*) values as being dark, normal, and pale during three independent trials. The limit values of L* to discriminate the three fillet colour groups (dark, normal and pale) were established considering the mean and SD of L*-distribution as follows: dark (L*< mean-SD), normal (mean-SD <L*< mean+SD), and pale (L*> mean+SD). For practical purpose, the limit values L*=50 and L*=56 were used to select the dark and pale breast meat, respectively (dark, L*<50; normal, 50<L*<56; pale, L*>56). Colour (L*a*b*) was measured on the medial surface and ultimate pH (pHu, probe method) was determined on the cranial section of each fillet. The right fillet from each breast was kept intact and used to determine drip loss (by calculating the loss of weight after 24h at 2-4°C) (Honikel, 1998), cooking loss (by cooking the samples on convection oven at 180°C until 72°C at core sample), moisture after cooking (AOAC, 1990) and shear value (Papinaho & Fletcher, 1996). The left fillet from each breast was ground and used to assess cooking loss and shear value on a meat patty as well as moisture after cooking. Furthermore, the water distribution in intact breast meat was studied by calculating the LR-NMR parameter $T2_a$ and $T2_b$ by using Minispec Bruker PC/20, according to the method previously described by Cremonini *et al*. (2001). ANOVA was performed with the GLM/SAS procedure testing both colour group and replication as main factors as well as the interaction term using residual error. When interaction was significant, the interaction mean square error was used to determine the significance of the main effects (SAS Institute, 1998). Means were separated using Newman-Keuls multiple range test.

Results and discussion

The histogram showing the L* value distribution in broiler breast meat during processing (Fig. 1) exhibited a mean L*-value of 52.98, SD 2.89, skewness –0.02 and kurtosis 0.70.

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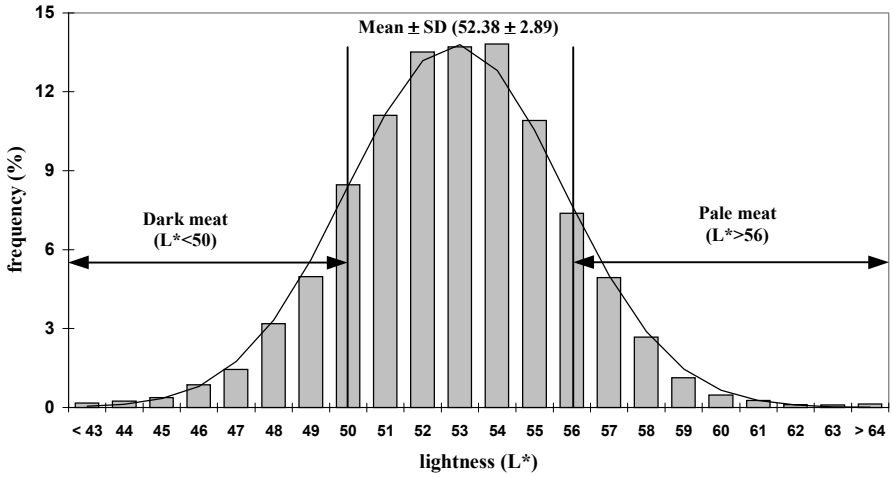
Figure 1 – Distribution of L* values in broiler breast muscle fillets (n = 6997).



The overall range in measured lightness was considerable and varied from 40 (dark) to 66 (pale). Moreover, the magnitude of the range found in this study was comparable to results found in North America (Barbut, 1997; Woelfel *et al*., 2002) and the United Kingdom (Wilkins *et al*., 2000). The overall distribution suggests that breast meat lightness in Italy is quite similar with that reported by Woelfel *et al*. (2002) and Wilkins *et al*. (2000) whereas it is considerably higher in respect with the findings by Barbut (1997). If the lightness limit values indicated by Barbut (1997) (L*>50) and Woelfel *et al*. (2002) (L*>54) to discriminate the pale breast meat were applied to the present study, the occurrence of pale meat with poor characteristics for further processed meat would be greater than 85 or 35%, respectively. This indicates that care must be exercised to use L* limit values established in different conditions. The effect of colour group on meat colour and ultimate pH are presented in Table 1.

Table 1 – Effect of colour group on broiler breast pH_u and colour lightness (L*), redness (a*) and yellowness (b*).

Group	n	L*	a*[1]	b*[1]	pH_u[1]
Dark	72	48.29±0.21[c]	2.96±0.07[a]	1.07±0.08	6.04±0.01[a]
Normal	72	53.51±0.18[b]	2.05±0.11[b]	1.52±0.09	5.89±0.01[b]
Pale	72	57.53±0.27[a]	1.63±0.12[c]	2.08±0.12	5.77±0.02[c]

[a-c]: Means within rows with differing superscripts are significantly different (P<0.01).
[1]: Significance determined by replicate mean square error as the statistic test.

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The L* and a* values were significantly different and consistent with the selection criteria by colour, whereas no differences were found in b* values. The pH_u values of dark, normal, and pale groups were significantly different from each other ($P<0.01$). These results confirm that higher muscle pH is associated with darker meat while lower muscle pH values are associated with lighter meat (Fernandez *et al*., 2001). The functional properties of dark, normal and pale intact and ground fillets are presented in Tables 2 and 3, respectively.

Table 2 – Effect of colour group on intact broiler breast meat characteristics.

Group	n	Drip loss (%)	Cooking loss (%)	Moisture after cooking (%)	Shear value (Kg/g)	$T2_a$ (ms)	$T2_b$ (ms)
Dark	36	1.73±0.09	18.84±0.43^c	68.54±0.16	3.36±0.33	82.72±2.70^c	38.34±0.36^c
Normal	36	1.56±0.08	21.13±0.51^b	68.36±0.18	3.36±0.30	106.77±3.37^b	41.02±0.30^b
Pale	36	1.68±0.10	23.84±0.51^a	68.30±0.18	3.48±0.25	125.79±4.19^a	42.36±0.29^a

$^{a-c}$: Means within rows with differing superscripts are significantly different ($P<0.01$).

Table 3 – Effect of colour group on ground broiler breast meat characteristics.

Group	n	Cooking loss (%)	Moisture after cooking (%)	Shear value (Kg/g)
Dark	36	21.02±0.36^c	67.87±0.19	2.05±0.05
Normal	36	24.66±0.31^b	67.35±0.27	1.94±0.05
Pale	36	27.69±0.40^a	67.31±0.31	2.09±0.04

$^{a-c}$: Means within rows with differing superscripts are significantly different ($P<0.01$).

The cooking loss of both intact and ground dark, normal, and pale groups were significantly different from each other ($P<0.01$). These results are consistent with previous studies (Qiao *et al*., 2001; Van Laack *et al*., 2000) and indicate that wide differences in raw broiler breast meat colour result in variations of WHC. These results are consistent with the NMR parameters $T2_a$ and $T2_b$ that were significantly ($P<0.01$) higher in pale breast meat. When the T2 increases, the water is less tightly bound to the meat structure. This condition has been associated with a lower WHC. A close relationship among NMR parameters and standard methods to assess WHC has been reported in earlier studies in poultry (Cremonini *et al.*, 2001; Cavani *et al*., 2002). However, the three experimental groups did not exhibited differences in drip loss. As discussed by Van Laack *et al*. (2000), the drip loss method was initially developed for pork and it does not seem very suitable to measure WHC in poultry. No differences were found in shear value and moisture after cooking. This study has provided the first

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comprehensive survey of the variability in broiler breast meat colour which exists within the portioned meat trade in Italy and indicates that a high incidence of pale broiler breast meat could exist. Furthermore, these results have stressed the necessity for each processing plant to determine its own lightness values for sorting pale meat. Moreover, it was confirmed that pale breast meat is associated with lower ultimate pH and poor water holding capacity. In addition, the LR-NMR technique confirmed to be a further tool to discriminate broiler breast meat properties for further processing.

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CHICKEN MUSCLE GROWTH IN RELATION WITH GENOTYPE - IMPLICATION FOR MEAT QUALITY

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Keywords: muscle, growth, IGF, meat, quality

Abstract

The present study was conducted to relate muscle growth and meat quality with the ontogeny of muscle fibres in two chicken genotypes selected or not for breast yield and against abdominal fatness, and with the expression of two stimulatory growth factors (IGF-I and IGF-II) in muscle. Higher breast meat yields in the selected genotype resulted from larger and longer muscle fibres with no change in their total number. In the leg, the *Sartorius* yield was higher in the selected chicken and this muscle also exhibited larger fibres with a drift toward a more oxidative typing. The breast muscle hypertrophy in the selected birds corresponded to higher IGF-I mRNA levels at 4 and 6 weeks of age, suggesting that this paracrine factor could partly explain the phenotypic differences between genotypes.

Résumé

L'ontogénèse des fibres musculaires et l'expression dans le muscle de deux stimulateurs de la croissance, IGF-I et IGF-II, ont été étudiées en relation avec les performances et la qualité des viandes de deux génotypes de poulets sélectionnés ou non pour les rendements en filet et contre le gras abdominal. Le rendement en filet supérieur chez le génotype sélectionné était du à des fibres plus grosses et plus longues, sans modification significative de leur nombre. Les animaux sélectionnés avaient aussi un rendement en *Sartorius* (muscle mixte de la cuisse) supérieur, ce muscle présentant à la fois des fibres plus larges et une typologie plus oxydative dans le génotype sélectionné. L'hypertrophie du filet des animaux sélectionnés correspondait à une expression supérieure d'IGF-I dans le muscle, suggérant un rôle de ce facteur paracrine dans le déterminisme de la croissance musculaire.

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Introduction

The poultry production uses chicken genotypes which differs on their growth performances and body composition. Recent studies (Le Bihan-Duval et al., 1999; Berri et al., 2001) showed that the technological quality of chicken meat is related to the muscle growth potential of birds. Indeed, the breast muscles of chickens selected for overall growth and/or specifically for breast yield exhibit a lower rate and extent of pH fall post-mortem, which were associated with improved water holding capacity compared to that of unselected control. The present study was conducted to relate these observations with the ontogeny of muscle fibre characteristics in two experimental genotypes selected or not for breast yield and against abdominal fatness, and with the expression of two stimulatory growth factors (IGF-I and IGF-II) in this tissue.

Materials and methods

Chickens were from two experimental genotypes of the Station de Recherches Avicoles (Nouzilly, France): one has been selected over 16 generations for body weight, breast yield and against abdominal fatness, the other maintained without selection (Le Bihan et al.,1998). After 15 generations, selected chickens exhibited at 6 weeks of age 6% higher body weight, 21% more breast yield, and 25% less abdominal fat. Five chickens from each genotype were collected at 5, 8, 11, 14 and 17 days *in ovo*, at hatching, at 2, 4 and 6 weeks of age, then were weighed. From 11 days *in ovo* the yields of breast and leg, and from hatching, the yields of *Pectoralis major* (PM, breast muscle) and *Sartorius* (SART, leg muscle) were determined.

The histological analysis of PM and SART muscles was performed from hatching and 2 weeks of age, respectively. In the SART muscle, the fibre typology was determined according to the ATPase at pH 4.1 and succinate dehydrogenase (SDH) activities. Fibres were classified according to their contractile and metabolic pattern into slow oxidative (βR), fast oxidoglycolytic (αR) and fast glycolytic (αW) (Ashmore and Doerr, 1971). The mean cross sectional area of muscle fibres was measured by using the RACINE application developed from the Visilog software (Noesis, France). The geometrical analysis (area, length, width, thickness) of the PM muscle between 2 and 6 weeks of age allowed to estimate an index of the total number of fibres in each muscle.

The expression of the IGF-I and IGF-II mRNA was quantified by real time RTPCR (Pfaffl, 2001) in PM and SART muscles collected from 14 days *in ovo* and 2 weeks of age, respectively.

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The effects of the age and of the genotype were tested by a two-way analysis of variance and the means were compared by the Newman-Keuls test (SAS, 1989).

Results and discussion

Chickens from the selected genotype exhibited a slight growth retardation before 17 days *in ovo*. Thereafter, the tendency reversed, and from 2 weeks of age the breast yield of the selected genotype was significantly higher, differences between genotypes increasing with age. Leg yields were the same in the two genotypes. While the relative development of leg was regular, that of breast was biphasic with a decrease between 11 days *in ovo* and hatching and an increase after hatch.

The higher breast development of selected birds corresponded to larger (+2 to +11%) and thicker (+14% to +24%) muscles and fibres with an increased diameter (Tableau 2). According to geometrical and histological measurements, we did not show any significant difference in total fibre number between the two genotypes. Therefore, the increased breast development in selected birds comes essentially from fibre hypertrophy both in length and diameter.

Despite a similar leg yield, the selected chickens exhibited greater SART yields, which corresponded to increased fibre diameter, especially of the slow red (βr) fibres. Therefore, selection induced a decrease in the relative area occupied by the fast glycolytic fibres (αw) whatever the bird age (Tableau 2). Moreover, in the selected birds, the relative area occupied by the slow red (βr) fibres increased with age at the expense of that of the fast oxidoglycolytic fibres (αr). These results suggest a shift toward a more oxidative typing of the SART muscle, which amplify with age in the selected birds.

The expression of IGF-I and IGF-II were higher before than after hatch in both PM and SART muscle. The IGF-I mRNA levels were higher in the PM muscle of selected birds at 4 and 6 weeks of age (Figure 1), when the differences in breast yield and fibre diameter were the highest between the two genotypes. However the selection did not alter the level of the IGF-II mRNA in PM muscle nor that of IGF-I and IGF-II in SART muscle.

Conclusions

The increase in muscle yield resulting from selection is mainly due to an increase in muscle fibre size in both diameter and length. This fibre hypertrophy was related to a higher expression of IGF-I in muscle. Selection for breast yield and against abdominal

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fatness would also affect yield and typing of the mixed leg muscle *Sartorius*, with a shift toward a more oxidative metabolism. By contrast, selection for leanness generally led to more glycolytic muscles and meat quality defects in pig and bovine. On the same chicken genotypes, we have previously shown that the meat of the selected chickens exhibited a lower rate and extent of pH fall compared to that of their unselected control. These characteristics were also associated with lower muscle glycolytic potential and improved water holding capacity, suggesting that selection for muscle yield can be achieved in poultry without deleterious effect on technological meat quality attributes.

Acknowledgements

The authors thanks T. Bordeau, B. Chevalier and N. Wacrenie-Ceré for their valuable technical assistance.

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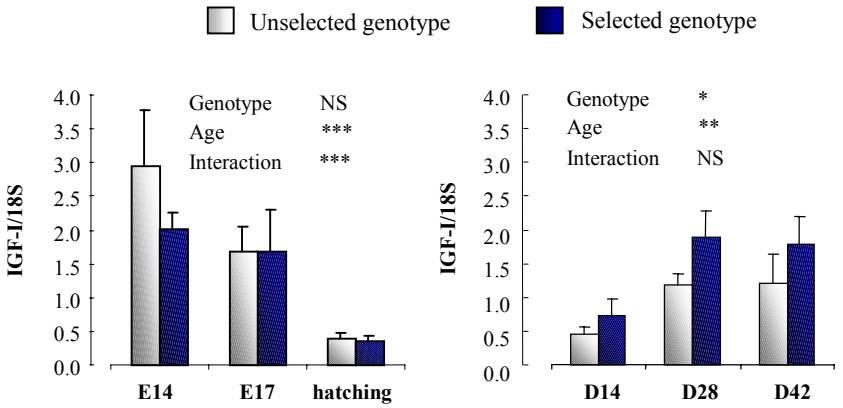
Table 1 : Cross sectional area (μm^2) of fibres in the *Pectoralis major* and *Sartorius* muscles (C: unselected genotype, S: selected genotype; ** P < 0.01, *** P < 0.001, NS: non significant)

Age	***Pectoralis major***		***Sartorius***					
	αW		αW		αR		βR	
	C	S	C	S	C	S	C	S
Hatching	91.3	104.2	-	-	-	-	-	-
2 weeks	334.8	378.0	220.5	309.5	200.7	319.7	107.2	175.0
4 weeks	820.2	986.4	632.0	790.2	608.8	802.6	404.0	620.4
6 weeks	1234.2	1535.0	541.0	728.4	611.4	846.4	432.2	679.6
Genotype	***		**		**		***	
Age	***		***		***		***	
Interaction	NS		NS		NS		NS	

Table 2 : Relative surface (%) occupied by the different types of fibres in the *Sartorius* muscle (C: unselected genotype, S: selected genotype ; * P < 0.05, NS: non significant)

Age	αW		αR		βR	
	C	S	C	S	C	S
2 weeks	61.1 %	54.6 %	26.7 %	33.3 %	12.2 %	12.1 %
4 weeks	60.0 %	54.3 %	29.2 %	30.1 %	10.8 %	15.5 %
6 weeks	60.2 %	53.8 %	24.5 %	24.8 %	15.3 %	21.4 %
Genotype	*		NS		NS	
Age	NS		*		*	
Interaction	NS		NS		NS	

Figure 1: Evolution of relative levels of IGF-I mRNA in the *Pectoralis major* muscle (18S rRNA was chosen as reference gene; E: day *in ovo*; D: day post-hatch)



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PRELIMINARY ANALYSIS OF SENSORY-MOTOR EVALUATION OF POULTRY MEAT DURING CHEWING

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Keywords : Chewing, M. masseter, meat poultry

Abstract

We present preliminary data on sensory evaluation of poultry meat by recording surface electromyography of M. masseter. Ten male and female subjects chewed meat samples from three poultry genetic types (COU NU, CABIR, and ROSS) produced, slaughthered and cooked under similar procedures. Contractions of left and right Mm. superficial masseter and anterior temporalis muscles were recorded, and three variables analysed for the first chewing cycle: duration of the bursts (s), mean voltage of bursts (V), and muscle work (V*s). As mastication progressed, the duration of the contraction and compressive work of M. masseter decreased steadily from the first to the last cycles. Mean duration and muscular work were significantly shorter for the meat sample from the COU NU lineage in both sexes of subjects. The mean voltage of bursts of M. masseter was significantly greater for male subjects and not different between poultry meats. The work of the muscle was significantly influenced by factors poultry meat and sex of the subjects.

Résumé

Nous présentons des données préliminaires sur l'évaluation sensorielle de la viande de poulet au cours de la mastication observée par EMG de surface. Dix sujets mâle et femelle ont mastiqué des échantillons de viande issus de trois types de poulets (COU NU, CABIR et ROSS) élevés, abattus et cuits dans les mêmes conditions. Les activités des muscles Mm. Masseter et temporalis ont été enregistrées et trois variables mesurées : durée des bouffées, amplitude moyenne et travail musculaire (V *s). Au

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cours de la mastication, la durée des bouffées diminue significativement du premier cycle aux autres. La durée moyenne et le travail musculaire sont significativement plus faibles pour la viande issue du COU NU. L'amplitude moyenne et le travail musculaire sont significativement plus grands pour les sujets mâles. Par contre, l'amplitude moyenne n'était pas influencée par le type de viande mastiquée.

Introduction

Appearance, flavour, texture and nutrition are the four principal recognized factors of quality for food (Bourne, 2002). The importance of textural properties in the sensory evaluation during chewing of the food is largely recognized. Chewing is a complex rhythmic response controlled by the brain stem (Lund, 1991) under sensory modulation based on the collection, analysis and interpretation of various sensory signals. This modulation mainly depends on the biochemical and structural characteristics of the masticated material (tissue). By examining the relationships from food structure to texture, Wilkinson et al. (2000) report that three senses (hearing, somesthesis, and kinesthesis) are involved in texture perception during food consumption involving biting, chewing, manipulation and swallowing. In chewing, each movement of the trophic system (jaws and tongue) is under the coordination of masticatory cycle produced by muscular contractions. The contractions are carried out by motoneuron bursts that result in breakdown of the food into particles of various size incorporated with saliva in a cohesive bolus transported into the digestive tractus during swallowing (Hiiemae and Crompton, 1985). . In the meantime, crushing of the food releases molecules producing various sensory inputs (i.e.gustatory and olfactory; Heath, 2002).

A large number of recent studies demonstrate that several structural properties of the meat a lot of produced by muscular tissue influence the masticatory process (Mathevon et al., 1995; Mathonière et al., 2000 Mioche et al., 2002; Hiiemae et al., 1996). To understand the sensory perception and evaluation of this type of food, a lot of standard non invasive methods including surface electromyography (EMG) have been used. Recently, Gonzalez et al. (2001) summarized several aspects of the contribution of surface electromyography in the sensory assessment of meat properties, and showed that some quantitative data on the relationship between chewing activities and meat texture have been reported through the literature. All these data are used for sensory evaluation termed "tenderness perception" and "tenderness assessment". A serie of authors (Gonzales et al., 2001) studied tenderness by measuring various parameters of surface EMG signals from adductor muscles of the mandible working during the closing phase of the masticatory cycle for various meats (beef, pork).

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The aim of this preliminary study was to investigate the sensory-motor control of the activity of M. masseter acting during the closing phase of chew. First, we analyze the crossed effect of poultry genetic type, sex of the subject and masticatory cycle on this activity. Second, we concentrated on the first chew cycle and tested the crossed effect of sex of the subjects and poultry genetic types.

Material and methods

Ten human subjects (6 females, 4 males) aged between 22-26 years
were involved in this study after a full dental examination to show that all had at least eight pairs of natural post-canines teeth. These subjects received five samples (2 cm^3) of the pectoral muscle from three different specimens. Muscle samples were cooked by using a microwave oven in 200 ml of water during 80s. temperature: 92°C). Before testing and chewing, the temperature of the samples was about 50°C. Subjects were asked to chew freely meat samples. Five whole masticatory sequences were recorded by subject for each poultry genetic type. Based on neck movement, deglutition was visually determined. The contractions of left and right Mm. superficial masseter and anterior temporalis muscles located by palpation when subjects clenched their teeth were recorded using surface EMG with a Myodata system (Fig. 1) and signals were analysed using the software Acqknowledge (Biopac Systems). In this study, we only analysed the contraction of the M. masseter. After rectification of EMG signals, three variables were analysed for the chewing cycles: (i) mean voltage of burst, (ii) duration of the bursts, (iii) muscle work calculated as the integrate areas of each burst of the sequence

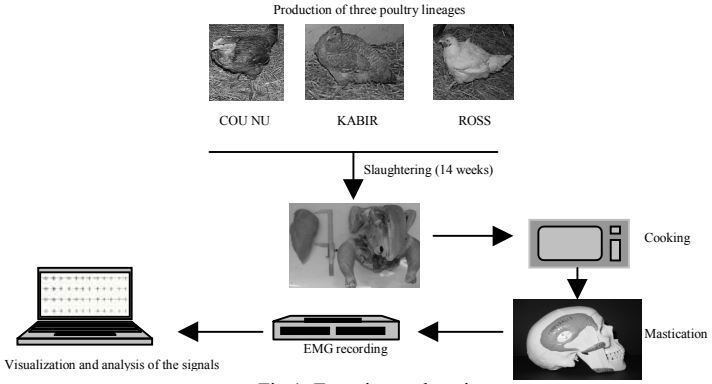

Fig 1. Experimental setting

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These values were collected from each muscle. All these data were statistically analysed using the Statgraphics version 5.0 plus (Manugistics, Inc, 2000). A total of 150 chewing sequences including a total of more than 1000 cycles were studied. Shapiro-Wilk's W-statistic for Normal distribution of the data and F_{max} –test for equality of variances assumed that values can be used for ANOVA. Two ANOVAs were calculated. First, we used a three-way ANOVA to test the significant difference in variables between the chewing cycle, the sex of the subjects and the poultry genetic type. F-statistics (Model I) for the fixed effects of cycle, sex and poultry lineages were obtained following the method described by Sokal and Rohlf (1995). The mean square of any source of variation is tested over the error mean square. The interactions between the three factors were included in the model of analysis. Student Newman-Keuls tests were used for comparing the differences of the mean when needed at level $P<0.05$. Second, we used a two-way ANOVA to test the significant difference between the meat of poultry lineages for the variables recorded from the muscular contraction during the first chewing cycle. The model (model I) included the sex of the subjects and meat issued from the three poultry lineages and their interactions. F-statistic for the fixed effect of meat was obtained by dividing the meat mean square by the mean square, and the F-statistic for the fixed effect of sex of the subjects by dividing the subject mean square by the mean square of the error (Sokal and Rohlf, 1995).

Results and discussion

All masticatory recorded sequences present a typical pattern shown in figure 2 for meat sample from ROSS poultry genetic type.

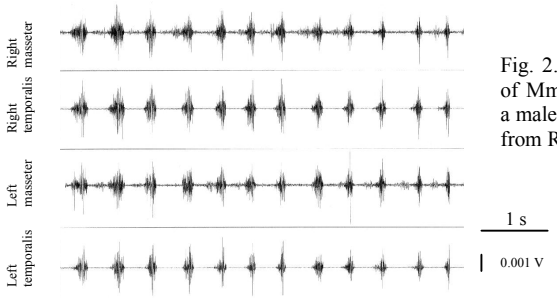

Fig. 2. Example of EMG recording of Mm masseter and temporalis for a male subject chewing meat sample from ROSS poultry lineage

As mastication progressed in both sexes of subjects, the duration of the occlusal activity of M. masseter decreased steadily from the first to the last chewing cycles. As shown by the Student Newman-Keuls test, the mean duration of first burst was

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significantly greater for the first chew than all the others for all the genetic types (Fig. 3).

In contrast, the mean amplitude and area of bursts were not significantly influenced by the position of the chew cycle along the sequences (Table 1). These data show that the duration of contraction of M. masseter at the first chewing cycle could be triggered by specific sensory inputs. These inputs could relate to the fact that subjects have to strongly modify the structure of the food at the first bite for facilitating teeth actions during the rest of the masticatory sequence. different sensory inputs from the buccal cavity could then play a major role as soon as the bolus begins to be shaped. For all cycles, the mean duration was significantly shorter in male subjects (male: 0.40±0.050 s; female: 0.44±0.007 s). The mean amplitude was greater for male subjects for all poultry genetic types (male: 7.24±3.64 E-5V; female : 5.65±3.08 E-5V). Poultry genetic type influenced the three muscular variables, and the crossed effect poultry genetic type x sex of subjects influences significantly mean voltage and work of the muscular contraction.

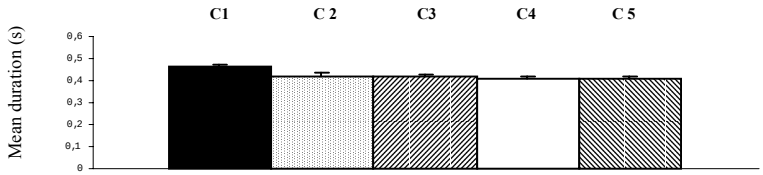

Fig 3. Mean (± standard deviation) of duration for the five first chewing cycles of M. masseter for pooled male and female subjects. C1-C5: first five chewing cycles.

TABLE 1. Summary F-statistics from three-ways ANOVA.

Variables	Cycle	Sex of subjects	Poultry genetic Types	A x B	A x C	B x C	A x B x C
Duration (s)	7.64*	4.7*	11.2*	0.68	1.53	0.49	1.1
Voltage (V)	0.54	7.4*	48.45*	0.08	0.24	11.33*	0.16
Work (V.s)	0.58	3.31*	27.12*	0.25	0.07	5.18*	0.22

*P ≤ 0,05

Then , we isolated the first cycle because it corresponds to the first bite that probably produces a major breakdown on unmodified sample whereas all following cycles relates to sensory feedbacks of modified meat to produce a bolus that looks like shaped minced meat (Mioche et al., 2002). All variables of the first burst were

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significantly different between male and female subjects (Tables 2, 3). But, the poultry genetic type had a significant effect on two characteristics of the EMG signal.
The mean duration was significantly shorter for the meat samples from the COU NU genetic type in both sexes as shown in figure 4. The work of chewing meat from COU NU was also smaller. The mean voltage and work of bursts of M. masseter was significantly greater for male subjects and not different between meat issued from the three tested poultry types (Tables 2, 3). The mean voltage and work were significantly influenced by the crossed factors meat and subjects (Table 2). These results show a main effect of sex of the subjects on EMG variables.

TABLE 2. Summary F-statistics from two-ways ANOVA. The first degree of freedom (d.f.) corresponds to fixed effect and the second to the error.

Variables	Poultry types		Sex of Subjects		Poultry types X Sex of subjects	
	d.f.	F	d.f.	F	d.f.	F
Duration (s)	2, 203	3.96*	1, 203	19.5**	2, 203	0..96
Voltage (V)	2, 203	1.66	1, 203	7.86**	2, 203	3.11*
Work (V.s)	2, 203	3.87*	1, 203	11.51**	2, 203	4.62*

*P ≤ 0,05 **P ≤ 0,01

TABLE 3. Mean and standard deviation for the three EMG variables recorded during first chewing cycle of male and female subjects.

Male Variables	COU NU			KABIR			ROSS		
Duration (s)	0.44	±	0.13	0.48	±	0.13	0.48	±	0.15
Voltage (V)	4.90E-05	±	1.30E-05	7.00E-05	±	3.00E-05	6.70E-05	±	3.70E-05
Work (V.s)	2.10E-05	±	1.40E-06	2.90E-05	±	1.20E-05	2.20E-05	±	2.00E-05
Female									
Duration (s)	0.39	±	0.09	0.52	±	0.14	0.46	±	0.11
Voltage (V)	4.50E-05	±	1.60E-05	6.30E-05	±	3.20E-05	5.40E-05	±	1.20E-05
Work (V.s)	1.20E-05	±	9.00E-06	1.80E-05	±	1.50E-05	1.30E-05	±	7.00E-06

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Probably, modulating the period of compressive time due to contact between the teeth of the lower and upper jaws and the food permits a change in the structural properties of the meat in relationship with its tenderness in both sexes. These results should be related to mechanical and structural properties of the samples obtained by dynamic measurements of meat of the three poultry genetic types.

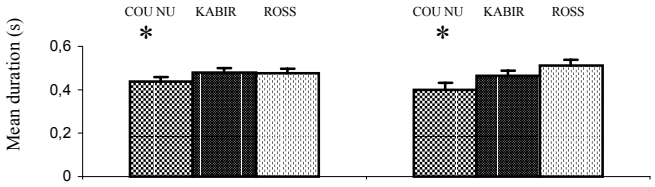

Fig 4. Mean (± standard deviation) of duration for the first chewing cycle of M. masseter in male and female subjects masticating meat samples from three poultry lineages. *:P<0.05

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USING A NOVEL RAZOR BLADE SHEARING METHOD TO MEASURE POULTRY MEAT TENDERNESS

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Keywords : poultry meat, texture analysis, sensory, tenderness

Abstract

This study was conducted to evaluate a new shearing method for the determination of poultry meat tenderness. Breast fillets were deboned at various times postmortem (0.25-24hrs) to yield a vast array of tenderness levels. A trained descriptive panel was used to evaluate samples for attributes including initial hardness (IH) and chewdown hardness (CH) while instrumental measurements included Allo-Kramer (AK) and razor blade (RB) shear and laser sarcomere length determination. The RB shear method exhibited a higher correlation to sensory attributes than the AK method suggesting that the new razor blade shear method is more advantageous in predicting poultry meat tenderness than the standard AK shear method. This new method not only has a higher sensory predictive value, but also requires shorter sample preparation time than the AK shear test as it is conducted on intact fillets.

Résumé

L'objectif de cette étude est d'évaluer une nouvelle méthode instrumentale pour mesurer la texture du filet de poulet. Les filets furent découpés de la carcasse à des temps variants de 0.25 à 24 heures post-mortem pour obtenir différents niveaux de tendreté. Un panel d'analyse sensorielle a évalué plusieurs indicateurs de texture tel que la fermeté initiale et après avoir maché l'échantillon 6 fois. Les tests instrumentaux comparés furent la cellule Allo-Kramer, la longueur des sarcomeres mesurée par la méthode de diffraction laser et une nouvelle méthode de cisaillement avec une lame de rasoir. Les résultats de la méthode de rasoir étaient davantage corrélés aux mesures sensorielles que ceux de la cellule Allo-Kramer. Ce résultat suggère que cette nouvelle méthode instrumentale est plus appropriée pour prédire la

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tendreté de la viande de volaille. Cette nouvelle méthode a également l'avantage d'être effectuée sans préparation de l'échantillon.

Introduction

Instrumental methods such as the Allo-Kramer shear compression system (multiple blade), Warner-Bratzler Shear Blade, and TPA (Texture Profile Analysis) are commonly used within the poultry industry for evaluating tenderness in broiler breast meat (Sams et al., 1990). Descriptive sensory analysis is a method that researchers commonly use for assessing attributes related to the tenderness of poultry meat. These types of tests are very reliable and have been shown to be correlated with instrumental analyses (White et al., 1964; Palmer et al., 1965; Lyon and Lyon, 1990; Lyon and Lyon, 1997); however, they can be expensive and exceedingly time consuming.
Research conducted by Cavitt et al. (2001) introduced a new instrumental razor blade shearing method for evaluating broiler breast fillet tenderness. Correlation coefficients of r=0.86 and r=0.88 were reported for maximum shear force and total energy of the razor blade shear test to that of the Allo-Kramer shear test, however no sensory data was available for comparison with razor blade shear data. Therefore, the objective of this study was to evaluate the effectiveness of various instrumental methods for predicting tenderness in broiler breast fillets and other sensory corollaries.

Material and Methods

Processing
A total of 270 commercial age broilers within three replications were slaughtered at seven weeks of age. Following a 10 h feed withdrawal period, birds were hung on a shackle line and commercially processed in order to obtain a varying range of tenderness in broiler breast fillets. Birds were electrically stunned (11 V, 11 mA, 10 s), manually cut, bled out (1.5 min), scalded (55 °C, 2 min), picked in-line and eviscerated. Carcasses were chilled at 13 C for 15 min followed by 1C for 45 min. After chilling carcasses were aged on ice until time of deboning at 0.25 h, 1.25 h, 2.0 h, 2.5 h, 3.0 h, 3.5 h, 4.0 h, 6.0 h, and 24.0 h postmortem (PM). The fillets were aged on ice until 24.0 h PM in plastic bags, and then stored at –20 C until evaluated.
Instrumental Measures
Sarcomere length was determined on the cranial region of the left fillets using the laser diffraction method. The right fillets and the caudal region of the left fillets were designated for instrumental and sensory measurements. Fillets were cooked according

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to Sams et al. (1990) to an internal end-point temperature of 76 C. Right fillets were cooled, stored overnight at 4 C, and then analysed for tenderness using the Allo-Kramer (AK) (10 blade) shear cell, and razor blade (RB) shear (8 mm width) method at 48 h PM. Following cooking the caudal region of the left fillets was immediately cut and served for trained sensory analysis.

Razor blade shear energy (N*mm) and shear force (N) was determined on intact fillets with an average height of 20 mm in duplicate at predetermined locations on each fillet. Shear energy and force were determined using a Texture Analyzer[1] with a razor blade (24 mm X 8 mm) set to a penetration depth of 20 mm. Crosshead speed was set at 10 mm/s. The razor blade shear force (RBF, N) was calculated as the maximum force recorded, while the razor blade shear energy (RBE, N*mm) was calculated as the area under the force deformation curve from the beginning to the end of the test. Samples for AK shear were excised from cooked broiler breast fillets. Allo-Kramer shear value (AKSV, kgf/g of meat) was determined on samples (40 x 20 x 7 mm) in duplicate. Shear values were measured using an Instron Universal Testing Machine[2] equipped with a 10-blade Allo-Kramer shear compression cell with a crosshead speed of 3.33 mm/s.

Sensory analysis was conducted by a six-member trained meat descriptive panel (Sensory Spectrum Inc., Chatham, NJ). Initial hardness (IH) was evaluated in the first bite stage while chewdown hardness (CH) was evaluated within the chewdown stage. All intensities were expressed to one significant digit on 15-point numerical scales. Panelists were randomly presented all treatment groups (16.5 cm cubes in soufflé cups) in duplicate during two testing sessions.

Statistical Analysis

The data for both instrumental and sensory analyses was subjected to analysis of variance (ANOVA) using the General Linear Models procedure (SAS 1999). Deboning time was defined as the main effect to determine the influence of deboning time on meat tenderness. For the sensory data, panelist was treated as random effect using Proc Mixed. Means were separated using Duncan's Multiple range test at a significance level of $P < 0.05$. The relationship between instrumental and sensory attributes analysed using PROC CORR in SAS (SAS 1999).

[1] Model TA-XT2i; Texture Technologies, Scarsdale, NY

[2] Instron Corp., Canton, MA 02021

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Results and discussion

Instrumental Analysis

Allo-Kramer shear values (AKSV) along with razor blade shear force (RBF) and energy (RBE) were used as instrumental measures of meat tenderness. Allo-Kramer shear value, RBF, and RBE values reported similar trends in tenderness at all sampling times (Table 1). An increase in AKSV, RBF, and RBE was noted from 0.25 h to 1.25 h. This result was somewhat expected due to the onset of rigor mortis development, which has been noted in previous research to have a direct influence on tenderness due to the fact that ATP is still present at time of sampling which initiates muscle shortening. Other than the initial increase, the AKSV, RBF, and RBE values generally decreased over time as expected. Lyon and Lyon (1990) reported similar results in poultry and Wheeler and Koohmaraie (1994) in lamb. Additionally, a second increase was noted from 2.5 h to 3.0 h (Table 1).

Descriptive Analysis

The nine PM deboning times provided a wide range of tenderness within the broiler breast meat as noted by statistically significant differences in instrumental analyses and descriptive analysis (Table 1). Initial hardness means followed a similar trend as the instrumental data in the development of tenderness with the progression of deboning time. Meat samples deboned at 0.25 h were not significantly different for initial hardness than those deboned at 1.25 h to 3.0 h PM. As deboning time progressed to 24.0 h PM, meat samples decreased in initial hardness values as anticipated, likely stemming from a progression of rigor mortis development and ATP depletion resulting in less muscle shortening following deboning. Results from the chewdown stage of evaluation yielded trends for chewdown hardness similar to those for initial hardness attributes and instrumental parameters. No significant differences were reported for CH for meat samples deboned from 0.25 h PM to 3.0 h PM. Similarly, meat samples deboned from 3.5 h PM to 24.0 h PM produced little variation in tenderness with sporadic significant differences being reported. The 6.0 h and 24.0 h PM treatments did not differ significantly from each other for any of the sensory attributes. However panelists were able to detect differences between meat samples deboned early (<2.5 h PM) and late postmortem deboned samples. The lack of significant differences between each of the sampling times could be attributed to increased variation between individual samples at each of the deboning times due to the biological nature in the onset of rigor mortis development.

Instrumental and Sensory data relationship

Both the instrumental and sensory tests were able to identify differences in the samples as a function of deboning time. The relationship between instrumental parameters and sensory attributes is presented in Table 2. Relationships between instrumental and sensory data proved to be highly correlated for such attributes as initial hardness and

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chewdown hardness. When comparing the various instrumental methods, the Allo-Kramer shear results exhibited the lowest correlation to sensory attribute ratings. In contrast, the sarcomere length and razor blade shear force and energy were better predictors of meat tenderness measured by sensory means. Razor blade energy also seemed to be a better predictor of sensory attributes than razor blade shear force.
The razor blade test provides obvious advantages over methods such as sarcomere length determination by laser diffraction and Allo-Kramer shear as it requires no sample cutting, nor is it excessively destructive as only a small incision is made in the sample. The razor blade test proved to also be less time consuming as 60 measurements can be performed in 1 hr compared to 30 measurements in the same amount of time for the Allo-Kramer shear test.

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Table 1: Mean instrumental and sensory texture measurements of broiler breast fillets deboned at varying times postmortem.

	Instrumental Analyses			Sensory Analyses	
Debone Time	AKSV (kgf/g)	RBF (N)	RBE (N*mm)	IH	CH
0.25	9.87^{b}	12.79^{ab}	165.78^{ab}	8.3^{a}	7.8^{a}
1.25	14.33^{a}	13.81^{a}	180.93^{a}	8.4^{a}	7.9^{a}
2.0	10.53^{b}	12.55^{ab}	164.58^{abc}	8.3^{a}	7.3^{ab}
2.5	7.22^{cd}	12.61^{ab}	159.59^{bc}	7.9^{abc}	7.3^{ab}
3.0	8.33^{c}	13.35^{a}	164.25^{abc}	8.0^{ab}	7.3^{ab}
3.5	6.40^{de}	11.82^{bc}	147.89^{cd}	6.8^{cde}	6.4^{bcd}
4.0	6.27^{de}	11.36^{bc}	142.81^{d}	7.1^{bcd}	6.6^{abc}
6.0	5.45^{e}	10.47^{c}	136.27^{d}	6.1^{de}	5.7^{cd}
24.0	5.35^{e}	11.59^{bc}	142.57^{d}	5.8^{e}	5.3^{d}

Means within column with no common superscript differ significantly ($P < 0.05$).
AKSV : AlloKramer Shear Value ; RBF : Razor Blade Force ; RBE : Razor Blade Energy ; IH : Initial Hardness ; CH : Chewdown Hardness

Table 2. The relationship (correlation coefficients[1]) between instrumental texture measurements and descriptive textural attributes.

	Sarcomere Length	AKSV	RBF	RBE
IH	- 0.93	0.82	0.86	0.92
CH	- 0.91	0.81	0.85	0.90
Sarcomere Length	---	0.91	- 0.85	- 0.94

[1]All correlations significant (P [illegible]

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DETERMINATION OF WATER HOLDING CAPACITY IN CHICKEN MEAT

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Keywords : Water holding capacity, chicken, methods

Abstract

Four different methods of determining water holding capacity (WHC) of meat were compared in chicken muscles. Variation in WHC was induced by supplementation of creatine (CG) and pyruvate (PG) to drinking water 42 hours before slaughter. In the breast muscle, methods were: a bag drip method, a modification of the bag drip method (EZ-method), a compression/filter paper method and application of low field H-NMR. Of the 'traditional' methods, the compression/filter paper method (relative weight loss) appear to be the most reliable. On the other hand, several of the attributes based on the NMR relaxation data showed significant differences between CG supplemented and control animals. Further validation of this method is needed before it can be practically applicable.

Résumé

Quatre méthodes de détermination du pouvoir de rétention d'eau (PRE) de la viande ont été testée sur les muscles de poulet. Des variations de PRE ont été induit par des supplémentation en créatine (CG) ou en pyruvate (PG) de l'eau de boisson des animaux 42 heures avant l'abattage. Pour le muscle du filet les méthodes utilisées étaient : la méthode d'égouttage en sac, une méthode modifiée de celle-ci, la méthode de compression/papier filtre, et la RMN à basse résolution. La méthode de compression/papier filtre s'est révélée la méthode « traditionnelle » la plus fiable. Par ailleurs certains paramètres obtenus par RMN mettent en évidence des différences significatives entre le groupe contrôle et supplémenté en créatine (CG). Cette dernière méthode doit maintenant être validée pour être utilisée en pratique.

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Introduction

PSE and a concomitant reduction in WHC is a growing problem in the poultry industry (Woelfel et al., 2002 in broilers and Sosnicki et al., 1998 in turkey) affecting yield, processing ability and general appearance of the meat (Van Laack et al., 2000). In poultry meat, several methods have been applied to determine WHC such as the bag drip method either performed on the entire breast muscle (Woelfel et al., 2002) or on slices of breast meat (Wynveen et al., 1999), a filter paper compression method (Urbin et al., 1962). These methods are generally adaptations of methods developed for WHC measurements in pork meat. Determination of WHC is generally subjected to very large variations, which is either due to methodological error or to the existence of large intra-muscular variations. Consequently it is recommended to use large samples and also to perform duplicate or triplicate when possible. None of these recommendations can be met when determining WHC in chicken muscles because of the small muscle size. From our experience, determination of WHC using the bag drip method is not a reliable method. We perform a survey on methods to determine WHC in search for an alternative and more reliable method for further refinement and validation for future use in chicken muscles. We present here the results of this survey. Although breast muscle is the most important muscle in the chicken economically, other muscles like the thigh also substantially contributes to the overall economy. As the breast muscle is characterised as exclusively consisting of white glycolytic type IIB fibres, and *M. Iliotibialis* of the thigh contains approx. 30% oxido-glycolytic fibres, different response to treatments of the two muscles might be expected.

Material and Methods

One hundred eighty chickens (Ross 208) were used in the experiment. The animals were placed in 9 rooms ($1.6m^2$). Standard procedures were used for feeding, temperature regulation and light exposure. Six days before slaughter a water tank was inserted in each room to get chicken used to drink water by this way. Forty two hours before slaughter three of the tanks were filled with water (control), three with a solution of water, glucose and pyruvate (50g/l and 9g/l, respectively) and three with a solution of water, glucose and creatine (50g/l and 9g/l, respectively). At the day of slaughter, all birds from the three rooms were caught, placed in boxes and transported to the slaughterhouse on site. The broilers were slaughtered room wise in a rolling order over the three days to compensate for differences in lairage time at the slaughterhouse. After slaughter, de-feathering, evisceration and chilling, the chickens were stored at 4^oC. For all WHC determinations sampling was performed 24 hours

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after slaughter. For the bag drip method, the *Pectoralis major* was excised and cut perpendicular to the fibre direction in the widest part. A slice of 1 cm was taken from the anterior part, then weighed (weight approx. 25 g), placed in a non-absorbing net mounted in a plastic bag and allowed to hang for 48 hours, then wiped of with a paper towel and weighed again. Water holding capacity is expressed in relative weight loss. A second slice was cut from the posterior part of the breast muscle. A unique sample from the centre of this slice was punched out in the direction of the fibres using a puncher with a diameter of 10mm. This sample was weighed and placed in a test tube closed with a lid to avoid evaporation. After storage of 48 hours at 4^{o}C reweighed and relative weight loss was used as expression of WHC (Rasmussen and Andersson, 1996). The controlateral muscle was excised and cut in the same way. A slice of 1 cm was taken from the anterior part and was used for determination of WHC by the filter paper/compression method. The sample was punched out from the centre as for the EZ-method. Initial weight was recorded and the sample was placed on filter paper (Whatman 41). On top of the sample was placed a glass and on top of that a weight of 60g, giving a total compression weight of 64.92g. Final weight was recorded and expressed in relation to initial weight. Digital photos were taken of the filters and total area of exudate was measured and likewise the area of the sample from a marked borderline on the filter paper. This was performed by image analyses (Image Pro, Cybernetics, Silver Spring, USA) using a borderline tracking facility. Total and relative area of exudate was used as indication of water holding capacity. From the posterior part a sample 1*1*5 cm was cut from the centre of the muscle in the fibre direction and used for NMR measurements. Immediately upon excision the sample was transferred to test tube, transported to the laboratory (within 15 minutes) then allowed to equilibrate to 25^{o}C in a water bath before measurement using a Maran Benchtop pulsed NMR Analyser (Resonance instruments Ltd, Witney, UK) using a magnetic field strengths of 0.47 Tesla and performed according to Bertram et al. (2002). Calculations included selected T_{21} and T_{22} parameters. Because *M. Iliotibialis* is much smaller than Pectoralis major,only two methods could be used for determination of WHC. The left muscle was excised and the sampling performed as for the EZ method except that punching was performed perpendicular to fibre axis due to the thickness of this muscle and was placed on a metal thread in the tube, so the edges were free and bend downward. The samples for NMR measurements were taken from the right muscle as described for the breast muscle.

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Results and discussion

The difference in WHC between the muscles using the EZ-method is shown in fig. 1.

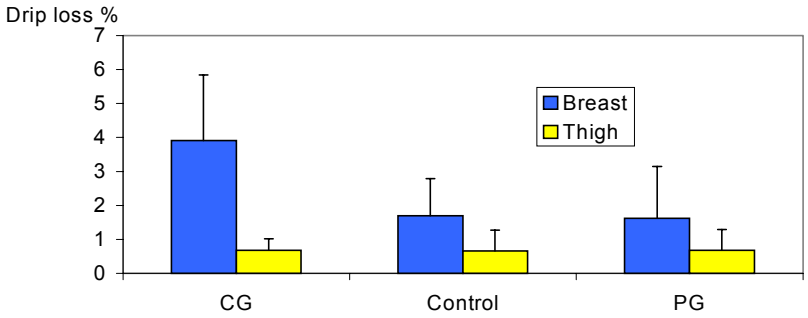

Fig 1. Mean values and SD of the two muscles using the EZ method

As expected creatine supplementation resulted in a significantly decreased WHC in the breast muscle, whereas no effects were observed in the thigh muscle. Pyruvate supplementation, however, did not increase WHC as it has been demonstrated in earlier experiments. SD is pretty high and CV in the groups amount to 49, 64 and 94% respectively for CG, control and PG in the breast muscle and of a similar magnitude for the thigh muscle. The difference between muscles does not represent a true difference as the sample for determination of WHC in the thigh muscle was cut perpendicular to the fibre axis resulting in less free cut surface of fibres and may therefore account for the major part of the difference, but of course difference in fibre type distribution cannot be ruled out. Figure 2 shows the mean value of the breast muscle drip loss measured by the four selected methods . All of these methods display a similar response to the treatments, and by none of them were observed differences between the control animals and the PG supplemented.

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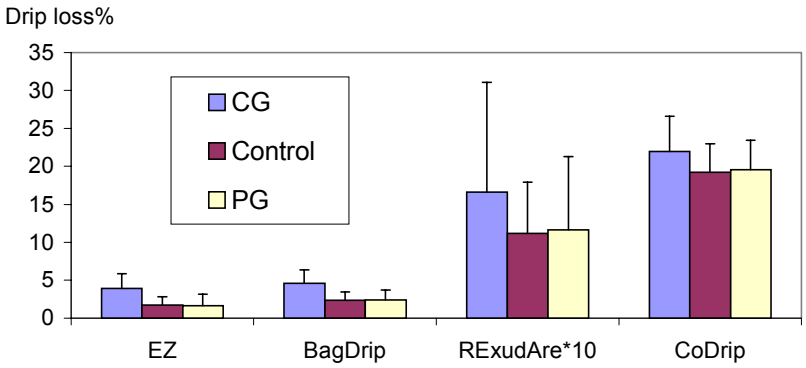

Figure 2. WHC expressed as drip loss (mean value and 1SD) in the breast muscle by four different methods.

Based on calculations of CV the filter paper compression/method (relative weight loss) appear to be the most reliable in terms of detecting differences between treatments. However, due to the extra compression force it represents an over-estimation of the true drip loss. The NMR T_2 relaxation decay in muscle tissue is multi-exponential, indicating the existence of different water populations in the muscle tissue. This characteristic has been used to determine WHC, and correlations between the slowest T_2 component (T_{22}) and WHC of pork has been shown by Tornberg et al. (1993). In the present survey we have calculated a number of characteristics (area, maximal amplitude, mean and standard deviation) from the relaxation data from the different components T_{2b}, T_{21}, and T_{22}. Twelve different parameters could significantly detect differences between the CG supplemented and the control animals in the breast muscle. Seven parameters ($T_{21\ mean}$, $T_{21\ top}$, $T_{21\ SD}$, $T_{22\ area}$, $T_{22\ SD}$ and the parameters derived by exponential fitting $T_{2(1)}$ and $T_{2(2)}$) could detect significant differences between these two treatments in the thigh muscle. Surprisingly two of the parameters also derived by bi-exponential fitting, the $T_{21\ int}$ and $T_{22\ int}$, only revealed significant differences between controls and CG in the thigh muscle but not the breast. At present the low field proton NMR relaxation appears to be the most promising of methods for determination of water holding capacity in chicken muscles, however, the mechanisms behind the distribution of water within the muscles and their importance to WHC are not fully understood.

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CONTENTS

THE EFFECT OF INCUBATION TEMPERATURE ON TURKEY MUSCLE DEVELOPMENT

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Keywords: muscle, myogenin, incubation temperature

Abstract

Embryonic temperature manipulation appears to have profound effects on the muscle potential of the turkey posthatch. Previous research (Somaiya A., PhD thesis, 2003) has shown that manipulating the incubation temperature (4 days at 38.5ºC or 35.5ºC) away from the control temperature (37.5ºC) causes a significant alteration in posthatch muscle (16 day poults). Eggs subjected to 35.5ºC from 5-8 embryonic days (ed) produced poults with significantly fewer myofibres. In contrast poults hatched from eggs incubated at 38.5ºC for 5-8 ed and 9-12 ed showed a significant increase in nuclei and fibre number respectively. Subsequent trials are ongoing and focus on embryonic parameters with manipulations at 5-8 ed. Myogenin, expressed during myofibre differentiation, showed a peak at ed16 for controls, whereas values for temperature treated embryos peaked at ed18. At ed18, 38.5ºC treated embryos showed a significant increase in expression. All results are in comparison to controls. These results indicate an apparent delay in differentiation of muscle in the temperature-manipulated embryos. From previous research we hypothesise that the improved muscle parameters will produce a better quality of meat. Ongoing research using Real-time RT-PCR will study factors to develop a clearer understanding of the action of incubation temperature on the development of embryonic turkey muscle.

Résumé

La température d'incubation embryonnaire joue un rôle important sur le potentiel musculaire de la dinde, après éclosion des oeufs. De précédentes recherches (Somaiya A., thèse de PhD, 2003) ont montré que changer la température d'incubation des oeufs

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de quelques degrés (4 jours à 38.5ºC ou 35.5ºC) par rapport à la température contrôle (37.5ºC) induit des changements significatifs sur le muscle après éclosion (dinde de 16 jours). Les oeufs incubés à 35.5ºC pendant les jours embryonnaires 5-8 (ed) ont produit des dindes qui avaient significativement moins de myofibres. Au contraire, les oeufs couvés à 38.5ºC pendant les périodes 5-8 ed et 9-12 ed ont présenté une augmentation significative du nombre de noyaux et de fibres musculaires respectivement. D'autres expériences en cours visent à manipuler la température d'incubation pendant les stages embryonnaires 5-8 ed. La myogénine, qui est exprimée pendant la différenciation des myofibres, atteint son maximum d'expression à ed16 pour le groupe contrôle, tandis que ce maximum est atteint au stade ed18 pour les groupes expérimentaux. L'expression de myosine est significativement augmentée au stade ed18 dans les embryons traités à 38.5ºC. Tous les résultats ont été comparés au groupe contrôle. Ces résultats indiquent un retard apparent dans la différenciation musculaire lorsque les embryons ont été incubés aux températures expérimentales. En reprenant les résultats de précédentes recherches, nous suggérons que l'amélioration des paramètres musculaires pourrait produire une viande de meilleure qualité. Des études actuellement en cours, utilisant la technique "Real time RT-PCR", permettront de mieux comprendre comment la température d'incubation agit sur le développement embryonnaire du muscle de dinde.

Introduction

Poultry meat is deemed to be the most popular animal food product worldwide (Mulder, 1997). A constant goal of the meat industry is to increase the quality of produce delivered to the table. Husbandry procedures and genetic lines are being improved all the time to achieve this objective.

Turkey muscle in meat-producing birds is composed of very large myofibres. These large fibres can lead to pathological problems. Birds with these big muscle fibres grow via hypertrophy, an increase in fibre size, rather than hyperplasia, an increase in fibre number.

Consider however the Norfolk Black Turkey, although a slow growing bird, its muscle is very fine and is almost certainly made of many small fibres, yielding tastier meat. Mammals that have many small fibres in their muscle produce tender cuts of meat (Crouse *et al.,* 1991). The question arises as to whether we can manipulate the fibre number in turkeys to produce the same effect.

Changing incubation temperature can alter muscle composition in trout and salmon. A decrease in incubation temperature causes growth by increase in muscle fibre number rather than size (Stickland *et al.,* 1988). This alteration is possibly brought about by a change in the myogenic regulatory factors that control muscle development. Previous

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research shows that a lower environmental temperature delays and prolongs myogenic regulatory factor expression and muscle differentiation in rainbow trout (Xie *et al.,* 2001).

Is it possible to use temperature to alter fibre number in turkeys? A problem, however, arises in turkeys due to the embryos sensitivity to temperature. Temperature is the single most important factor affecting hatching success (French, 1994). The optimum incubation temperature is 37.5ºC (Christensen and Bagley, 1987); any temperature outside of this for a prolonged period can affect hatchability and development of the embryo. However research has proved that applying a temperature of 38.5ºC to the eggs for 3 or less days has no detrimental effect, whereas exposure for 5 or more days significantly decreases hatch (French, 2000). Work was therefore carried out within these confines.

Our aim was to establish whether changing incubation temperature for short periods affects muscle composition in the turkey, and, to determine the most influential time point of temperature manipulation. We hypothesise that an alteration in incubation temperature will have an impact on muscle nuclei and muscle fibre number.

Materials and Methods

Experiment 1: To assess the effect of prehatch incubation temperature on posthatch growth, B5FLX eggs (British United Turkeys Ltd, BUT) were incubated at a control temperature of 37.5ºC. Eggs were subjected to a temperature regime of either 38.5ºC or 35.5ºC at time periods of 0-4 embryonic days (ed), 5-8 ed, 9-12 ed, 13-16 ed, 17-20 ed or 21-24 ed and were then returned to the control temperature. Eggs hatched and poults were reared until 16 days posthatch. Poults were killed and the *M.semitendinosus* dissected out. Slides were stained with H&E and assessed for fibre number and nuclei number per cross-sectional area using the KS300 Kontron Image Analysis system.

Experiment 2: To assess the prehatch influence of incubation temperature on prehatch muscle development, B5FLX eggs (BUT) were incubated at a control temperature of 37.5ºC. Eggs were subjected to a temperature regime of 38.5ºC or 35.5ºC at 5-8 ed and subsequently returned to 37.5ºC. Embryos were sampled at ed8, 14, 16, 18, 21, 23 and ed25. Legs were dissected. After sectioning, sections including the *M.semitendinosus* were antibody stained for myogenin (Santa Cruz), a muscle differentiation factor.

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Results

Eggs incubated at 38.5ºC at 5-8 ed produced poults at 16 days posthatch which exhibited a significant increase in nuclei number per cross-sectional area of the *M.semitendinosus* (figure 1). Changing the incubation temperature at any other time had no significant effect on the nuclei number.

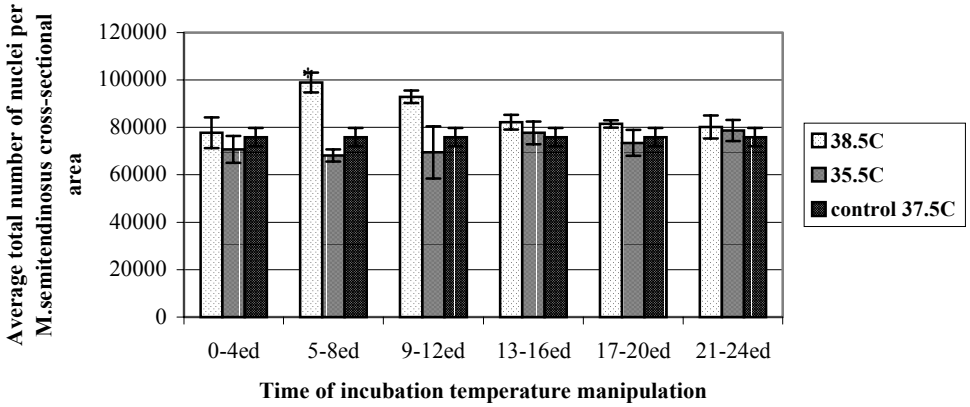

Figure 1: Variation in nuclei number within the *M.semitendinosus* of poults at 16 days posthatch having undergone an incubation temperature regime.

Eggs incubated at 35.5ºC at 0-4 and 5-8ed produced poults with significantly fewer myofibres at 16days posthatch than controls. Eggs incubated at 38.5ºC at 9-12 ed produced poults with significantly more myofibres.

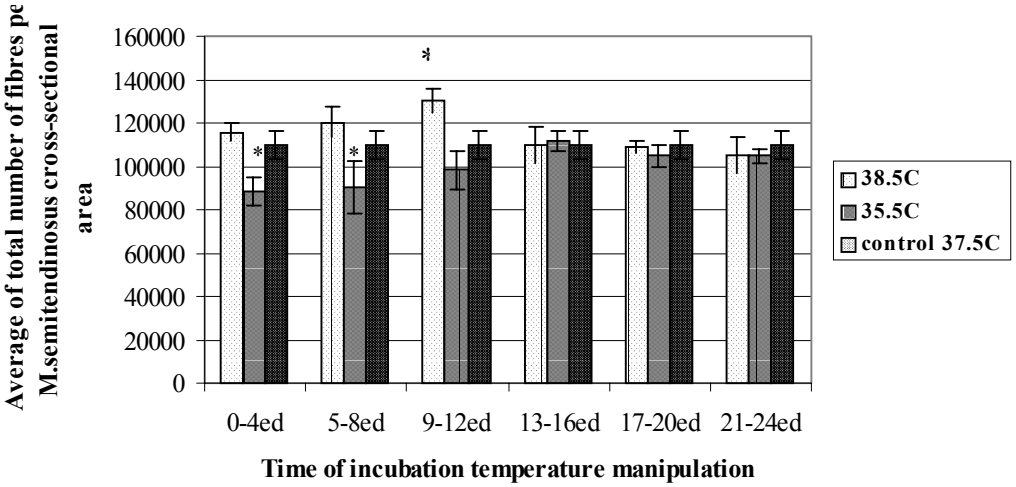

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Figure 2: Variation in fibre number of the *M.semitendinosus* in 16 day posthatch poults having undergone an incubation temperature regime.
Embryos undergoing a temperature manipulation at 5-8 ed showed a delay in differentiation of muscle formation. Myogenin levels for the temperature-manipulated embryos peaked at ed18, whereas levels for controls peaked at ed16. A significantly increased expression of myogenin was observed at ed18 from embryos incubated at 38.5ºC compared to controls and those at 35.5ºC.

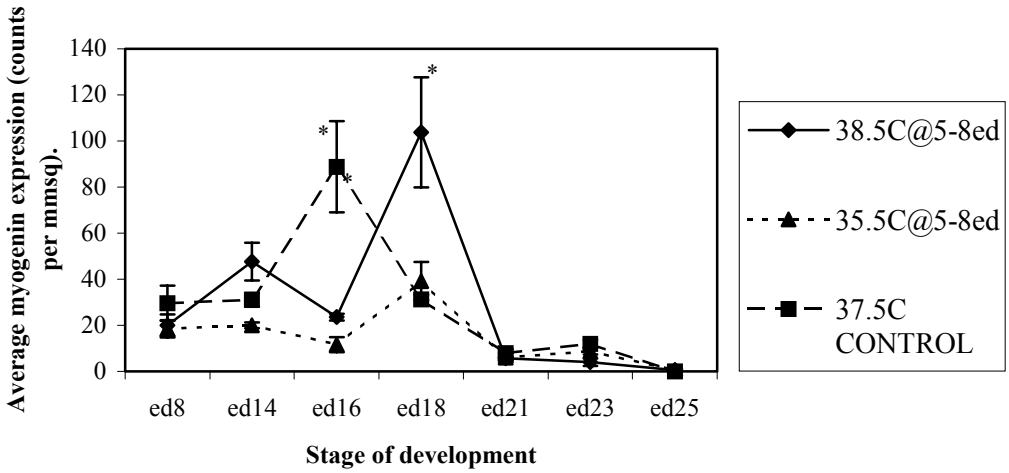

Figure 3: Myogenin expression in embryos when subjected to an incubation temperature manipulation.

Discussion

These results indicate that changing the incubation temperature for a four-day period in early incubation causes a change in muscle parameters posthatch. A higher incubation temperature of 38.5ºC produces an increase in fibre and nuclei number in the posthatch bird. A reduction in incubation temperature causes a decrease in fibre number. The time periods having the most influence occur within the stage of primary and secondary fibre formation in avian species, ed3-7 and ed8-15 respectively (Stockdale and Miller, 1987).
We speculate that an increase in fibre and nuclei number arises from a delay in muscle differentiation, allowing a greater period of proliferation to occur.

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Mammalian research shows that an increase in myofibre number aids the quality of meat obtained (Crouse *et al.,* 1991). We speculate that this increase in incubation temperature at 9-12ed, contributing to an increase in muscle fibre number, will cause a similar effect in turkeys as mammals.
Future research will ascertain meat quality in birds ready for the table and concentrate on the embryonic mechanisms via which this change in phenotype is occurring.

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CONTENTS

INVESTIGATIONS OF MEAT QUALITY OF M. PECTORALIS SUPERFICIALIS IN MALE AND FEMALE JAPANESE QUAILS (COTURNIX JAPONICA)

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Keywords: Japanese quail, carcass quality, meat quality, muscle fibre

Abstract

In this investigation the meat quality of Japanese Quails (Coturnix japonica) with special attention to poor meat quality was evaluated. The distinct sex-dimorphism was considered in experimental setup. Forty Japanese Quails (n=20 per sex) were pairwise fattened in cages. At the age of 170 days, the quails were slaughtered and early post mortem characteristics of meat quality measured. Specimens were taken from the M. pectoralis superficialis to investigate the structure of muscle fibres by histological-histochemical methods. There were significant differences between the sexes in regard to liveweight, weight and proportion of breast muscle, protein and fat content and colour value a* and brightness L* of meat. Increased carcass weights and higher proportions of breast muscle were related to higher crude protein and lower fat contents. The content of crude protein was positively correlated with an enlargement of muscle fibres. This effect was stronger in male quails. Generally, the breast muscle of quails is made up of only 13 to 14 % of white (glycolytic) muscle fibres. There is a tendency for female quails to have thicker breast muscle fibres compared to the males. On the whole, the condition and quality of meat from breast muscle of Japanese Quails can be characterized as good.

Résumé

Dans cette étude, la qualité de la viande de caille (Coturnix japonica) a été examinée en prêtant une attention toute particulière aux défauts éventuels. Quarante cailles japonaises (n = 200 par sexe) ont été élevées par 2 en cages. A l'âge de 170 jours, les

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cailles ont été abattues et des indicateurs précoces de qualité de viande ont été mesurés. Des échantillons de M. Pectoralis superficialis ont été prélevés afin d'analyser la structure des fibres musculaires avec des méthodes histochimiques. Le sexe a un effet significatif sur le poids vif, le poids et le rendement en filet, la teneur en protéines et lipides, la luminance et l'intensité de rouge de la viande. L'accroissement du poids carcasse et du rendement en filet est lié à une augmentation de la teneur en protéines et une diminution de la teneur en lipides de la viande. La teneur en protéines est corrélée positivement avec la taille des fibres musculaires. Cette corrélation est plus élevée chez les mâles. Le muscle du filet de la caille contient 13 à 14 % de fibres musculaires blanches (glycolytiques). La taille des fibres musculaires mesurée chez les femelles serait inférieure à celle mesurée chez les mâles. Dans l'ensemble, la qualité de la viande du filet de cailles Japonaises peut-être considérée comme bonne.

Introduction

In the economically important poultry species as turkey and chicken, meat quality alterations occured during the intensive selection on high end-weights. It is therefore of interest, whether and to what extent other poultry species could be negatively influenced by a similar selection strategy and wich role does the sex of the quails play in this direction. On this background, 40 Japanese Quails (20 ♂, 20 ♀) were examined on possible meat quality problems.

Material and Methods

In the investigation, 40 Japanese Quails (Coturnix japonica, n=20 per sex) of an heavy line were used. These birds were selected for life-weight over 14 generations. The rearing till the 14.th day happened in groups of 100 birds/m2 , from 15th to 35th day in groups of 90 birds/m2. They were fattened in pairs in pages, the temperature decreased from 37°C at the first day of life to 23°C after the 14th day (1°C per day). In the first three weeks, light was given 23 hours, in the next two weeks for 14 hours.
During the first four weeks of life, the quails were fed with turkey-feed (11,6 MJ ME, 29% crude protein, 0,6% methionine). Between the 28th and 35th day of life, they got chicken-feed with 13,2 MJ ME, 21,5% crude protein, 0,45% methionine. Till the 170th day of life, the quails got feed for laying hens (11,4 MJ ME, 17% crude protein, 0,37% methionine/Lysine.

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After stunning and draining blood from the quails, pH-value, L*,a*,b* and the conductivity were measured. For the histological investigation, specimens of the left m.pectoralis superfic. were taken and conserved in liquid nitrogen. The rest of both mm.pect superfic. was ground and frozen (-20°C) for the determination of the nutrients (§ 35 LMBG, Weender Analysis).
In the histological-histochemical treatment of the specimens (HORAK, 1983), the oxydative and the glycolytic muscle fibres were colourized different. The histological pictures were evaluated by an analytic software, developed by NIKON.

Results and discussion

For all investigations, the large breast muscle (M. pect. Superfic.) was used. The percentage of the breast muscle was with 26 % similar to other chicken-like birds (PINGEL, 2002). Turkey and chickens (meat type) sowed percentages between 25 % and 35 %. The histological investigation revealed, that the main type of muscle fibre was the oxydative type (85%), like in other animals, the muscle fibres of the glycolytic type were much bigger than the fibres of the oxidative type (Tab.1). With nearly 85 oxidative muscle fibres, quails have a much higher percentage compared to other poultry types (excepted ducks). Turkey and chicken possess nearly 100 % glycolytic fibres. The cross section areas of the glycolytic muscle fibres were with 3832 μm^2 higher than in chickens. Only turkeys had bigger glycolytic cells with till 7000μm^2 (KNUST, et al. 2000, WICKE, et al. 2001).
No cell-degenerations could be found in the M.pect. superfic. of the quails.

Tab. 1:
Muscle fibre characteristics of the breast muscle of the Japanese Quail (n=40).

Cell-Parameters	$\bar{x}$	s	SE
Cell-Area F/STO (μm^2)	956.5	199.4	31.5
Cell-Area FTG (μm^2)	3832.0	1034.8	163.6
Ø F/STO (µm)	35.0	4.6	0.7
Ø FTG (µm)	70.0	12.9	2.0
F/STO %	84.5	8.4	1.3
FTG %	15.5	7.0	1.1

(s: Standard difference; SE: Standard error)

Tab.2 shows the relationship between the quail weights and the characteristics of the meat quality. 20 minutes post mortem, a pH-value of 6.3 was measured in quails.

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Higher values could be found in chickens with pH-values of 6.41. The other poultry types turkey and duck had lower pH-values from 6.11 and 6.02, respectively (MAAK etal. 2002). Higher carcass-weights were positively correlated with conductivity values of the breast muscle (one indicator for cell damage). Hypertrophy of muscle cells, even of the glycolytic type, was found, too. Reason for the high conductivity values is the fact, that the big glycolytic cells in case of an possible damagerelease more cell fluid into the sorrounding tissue, than smaller cells could do. The fat content of the breast muscle decreases with higher breast muscle weights, especially in higher breast muscle portions (in %).

Tab.2:
Interactions between selected parameters of carcass composition and parameters of the breast muscle of the Japanese Quail (n=40).

Parameters of breast muscle	Carcass-weight (g)	M. pect. superfic. (g)	Breast muscle (%)
Protein %	-0.18	0.22	0.44*
Fat %	0.16	-0.36*	-0.63*
Ashes %	-0.04	-0.06	-0.04
pH_{20min}	0.06	-0.04	-0.11
$LF_{20min\ (mS/cm)}$	0.38*	0.43*	0.32*
L*	0.11	0.23	0.23
a*	-0.08	-0.29	-0.33*
b*	-0.11	0.02	0.12
Cell-Area F/STO (μm^2)	0.27	0.42*	0.35*
Cell-Area FTG (μm^2)	0.34*	0.47*	0.37*
F/STO %	0.03	0.01	-0.02
FTG %	-0.05	-0.07	-0.05

* $p \leq 0,05$

Tab.3 shows, that there is a relationship between the muscle contents fat and protein and some meat quality parameters. A higher fat content leads to darker meat with pronounced red colour, a higher protein content leads to brighter meat with a small tendency to green colour.
Even the histological parameters were influenced by the meat contents. A higher protein content is positively correlated with the diameter of the oxidative type fibres. With increasing fat content, the cell-areas of both muscle fibre types are decreasing. In selection for higher end weights, normally the cells become larger, but not the number of the cells raises; an effect, wich decreases the meat quality. Another aspect is the decreasing fat content with raising size of muscle cells. By strong selection on high carcass weights, the fat content decreases, with negative effects on meat quality. Fat is important for the taste of the meat. Because quails are marked as speciality, breeders should keep this in mind.

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Tab.3:
Correlations concerning the muscle contents of the Japanese Quail (n=40).

Parameter	Protein	Fat	Ashes
pH_{20min}	0,15	0,04	0,04
$LF_{20min\ (mS/cm)}$	0,01	-0,24	0,07
L*	0,50*	-0,49*	0,10
a*	-0,54*	0,57*	-0,19
b*	0,07	0,03	-0,10
Cell-area F/STO (μm^2)	0,29	-0,28	0,02
Cell-area FTG (μm^2)	0,23	-0,36*	0,07
Ø F/STO (µm)	0,32*	-0,32*	0,08
Ø FTG (µm)	-0,16	0,30	0,12

* $p \leq 0,05$

In contrast to turkey and chicken, the female quails reach higher end weights (Tab.4) than the males (PRZYWAROWÀ et al. 2001). Reason is the high weight of the sexual organs with 10-12 % of live weight (GERKEN 1989). Even the breast muscle weights were higher in female quails, compared with the male. The hens got significant higher protein contents and lower fat contents, than the male quails. There were no differences in histological parameters between the sexes measured. The crude protein content of the M. pect.superfic. of female quails is significantly higher than in the male quails. In contrast to the crude protein, the fat content of M. pect.superfic was significantly higher in male quails, compared to the hens. The meat of the large breast muscle was significant darker and more red coloured in male quails. No significant difference could be found between the sexes with regard to the muscle cell parameters. Investigations of the relationship between the parameters of weight and the meat quality parameters per sex have shown, that in male quails higher breast muscle weights led to higher conductivity values. In contrast to this finding, the conductivity of the breast muscle was positively correlated in female quails with breast muscle percentage. In opposite to male quails, a positive correlation between the percentage of breast muscle and the cross-sectional areas of oxidative muscle fibres could be found in quail hens. Only in male quails the slaughter weight and the cross-sectional areas of glycolytic fibres were positively correlated.

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Tab.4:
Carcass composition and meat quality parameters of the Japanese Quail, depending on sex (n=20 per sex).

Parameters	$\bar{x}$ ± SE ♂	♀	Significance
Liveweight (g)	334.1 ± 4.15	358.8 ± 5.46	***
Carcass weight (g)	207.1± 2.90	203.0 ± 3.85	n.s.
M. pect. superfic. (g)	51.2 ± 1.42	55.8 ± 1.42	*
Breast muscle %	24.7 ± 0.44	27.4 ± 0.44	***
TS (% M. pectoralis superfic.)	30.0 ± 0.27	28.5 ± 0.23	n.s.
Protein (% M. pectoralis superfic.)	23.9 ± 0.13	24.4 ± 0.10	**
Fett (% M. pectoralis superfic.)	4.4 ± 0.31	2.6 ± 0.19	***
Ashes (% M. pectoralis superfic.)	1.4 ± 0.02	1.4 ± 0.01	n.s.
pH_{20min}	6.2 ± 0.04	6.3 ± 0.04	n.s.
$LF_{20min\ (mS/cm)}$	2.1± 0.08	2.0 ± 0.07	n.s.
L*	38.9 ± 0.37	40.0 ± 0.33	*
a*	12.4 ± 0.43	10.9 ± 0.41	*
b*	2.0 ± 0.17	2.5 ± 0.17	n.s.

*p ≤ 0,05; **p ≤ 0,01; ***p ≤ 0,001 (SE: Standard error)

In the further literature, sex dimorphism in quails was described only in the weight parameters (GERKEN 1989). In Tab.5 the relationship between the muscle contents fat/protein and other muscle parameters could be shown only in male quails. Remarkable is the correlation between fat-/proteincontent and brightness values (L*) and coloration values (a*). Even a positive correlation between fat and protein content and some histological parameters (Protein⇔oxidative muscle fibres) could be described only in male quails. A higher fat content was positively correlated with a higher number of glycolytic fibres in the male breast muscle.

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Tab.5:
Interactions between the breast muscle contents and selected parameters of the meat quality of Japanese Quails, depending on sex (n=20 per sex).

Parameters	Protein		Fat		Ashes	
	♂	♀	♂	♀	♂	♀
pH_{20min}	0,33	-0,07	0,01	0,13	0,00	0,11
$LF_{20min\ (mS/cm)}$	0,29	-0,19	-0,45*	-0,40	0,11	0,06
L*	0,58*	0,18	-0,64*	0,08	-0,06	0,35
a*	-0,67*	-0,15	0,78*	-0,05	-0,11	-0,27
b*	-0,29*	0,14	0,47*	0,04	-0,30	0,14
F/STO cross section area (μm^2)	0,39	0,18	-0,41	-0,16	0,03	-0,02
FTG cross section area (μm^2)	0,38	-0,11	-0,50*	-0,03	0,21	-0,15
% F/STO	0,53*	-0,02	-0,50*	-0,15	-0,01	-0,39
% FTG	-0,36	0,01	0,47*	0,33	-0,01	0,36

*$p \leq 0,05$

Generally, the condition and quality of meat from breast muscle of Japanese Quails can be characterized as good.

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CONTENTS

EVALUATION OF NORMAL, PSE-AND DFD-LIKE CONDITION IN DRAKES' MUSCLES FROM THREE POLISH FLOCKS.

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Keywords : drake, PSE-, DFD-meat, pH, colour, muscles parameters

Abstract:

The aim of the study was to investigate whether drakes' breast and thigh muscles from P33, A3, K2 conservative flocks reveal PSE-and/or DFD –like condition in their meat. The pH and colour parameters (L*, a*, b*) respectively measured at 15 min. and 24 h post-mortem were determined as well as the intensity of colour by sensory panel at 24 h post-mortem. It was established that none of investigated trials demonstrated either the PSE-or DFD-like condition.

Résumé

Cette étude vise à tester la présence dans les troupeaux A3, P33 et K2 de muscles de type PSE ou DFD . Le pH et les paramètres de couleur (L*, a*, b*) des muscles du filet et de la cuisse ont été mesurés à 15 min. et 24hpost-mortem respectivement. L'intensité de la couleur a été déterminée par analyse sensorielle à 24hpost-mortem. Aucun des muscles analysés n'a pu être qualifié de type PSE ou DFD.

Introduction

For a long time , Genetic selection, control of nutritional and rearing factors have focused on the improvement in performance and body conformation of poultry. This aimed at increasing meat yield and lowering fat content. The increasing in meatness as well as rearing conditions, nutrition, handling and slaughter related processes could

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affect the chemical composition of muscles, their structure and yield, as well as the mechanisms which intervene when muscles become meat (Berri 2000). It is well documented that stress affects the quality of the meat. This effect depends on both duration and intensity of stress period. After death, as an effect of muscle glycolysis, pH decreased from about 7 to 5.4. pH decreases until glycogen reserve has been consumed or until pH reaches 5.4 which inhibits further glycolysis. The rate and the extent of pH decline are higher in glycolytic than in oxidative muscles. Chronic or exhaustion stress before death of an animal results in lower glycogen content in muscles and it entails high final pH and rigor shortening. The high final pH could produce dark, firm and dry meat (DFD) with short shelf life. This type of muscles appears mostly in cattle. On the other hand the high temperature or acute stress acting shortly before slaughter speeds up glycolysis and rigor mortis, and it is reflected by a faster drop in the pH. The accelerated rigor could lead to production of pale, soft and exudative meat (PSE), when the animals are not resistant to stress. This type of meat associated with poor functional properties of meat has been well explored in pigs.

A lot of authors have reported for a long time that turkeys and chickens are susceptible to stress, and the PSE-like condition in muscles have appeared especially in breast muscles. It is a big problem because 5-40% of those birds exhibited this anomaly (Berri 2000, Kijowski 1999).

The DFD-like condition in chicken muscles was found too by Kijowski and Niewiarowicz (1978), and Niewiarowicz et al. (1980). It took place when the pH_{15} values (measured 15 min. after slaughter) ranged from 6.4 to 6.6. On the basis of the investigation conducted by Knust and Pingel (1992) , Knust et al. (1995) assumed that the PSE - and DFD - like condition could appear in ducks' muscles. The authors stated that heat stress led to a faster pH decline in breast muscles of Pekin ducks at the 7 week of age and that cooked muscles were more tough . However, the feed and water deprivation led to higher pH values, particularly in thigh muscles. On the other hand Niewiarowicz et al. (1980) did not observe PSE- or DFD-like condition in Pekin ducks' muscles despite the diversity of glycolysis rate.

It is the reason why we conducted investigation on this topic. The contribution is a part of work on the evaluation of nutritive, technological and sensory values of Polish conservative ducks' flocks.

This study aimed at investigating whether drakes' breast and thigh muscles from A3, P33 and K2 conservative flocks reveal PSE –and /or DFD-condition.

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Material and methods

Drakes from three conservative groups of ducks maintained in situ in Waterfowl Breeding Department Dworzyska near Poznań of National Institute of Animal Breeding at Kraków and specified below were used as experimental material:
-Mini -ducks (K2), bred from wild duck (Anas platyrhynchos L) and Pekin ducks (FAO 2000)- 50 heads ;
-Polish Pekin (P33), a native breeding strain taken from a liquidated farm at Borowy Młyn in 1978 (FAO 2000)- 50 heads ;
-Duck population A3, progeny of a commercial stock imported from England in 1977- 56 heads.
During the testing period, the birds from all conservative groups were kept in one windowless poultry house with controlled environment and without access to a range. Ducks were reared up to the 4^{th} week of age in a poultry house of controlled air temperature, and afterwards were kept on range of restricted area , shedded and covered with straw. Birds were fed ad libitum on a compound feed mixture containing up to 20% crude protein and up to 12.13 MJ metabolizable energy in 1kg of feed.
From each of duck groups six males having body weight close to the arithmetic mean (K2- 1582g, v% 9.0; P33- 2342g, v% 7.9; A3- 2475g, v% 7.6) of sex within a given group were taken for study at seven weeks of age.
The colour parameters L* (lightness), a* (redness) and b* (yellowness) were measured with CHROMAMETER Minolta CR-200b.
The sensory assessment of colour was conducted by a sensory panel (8 trained persons), according to ANALSSENS NT programme using 10 point scale of intensity and expressed in conventional units.
The pH was measured with a digital pH-meter with a dagger electrode.

Results and discussion

Nowadays there are three commonly used indicators to detect the PSE or DFD type of meat : lightness, pH and electrical conductivity. Among them the pH_1 and pH_2 measurements have to be defined . In case of pigs and cattle the pH_1 value is usually measured at 45 min. after slaughter. However in case of poultry the reference post-mortem time for pH_1 varied according to the different authors, : 15 min. (Kijowski and Niewiarowicz , 1978), 20 min. (Barbut et al. 1995), 22 min. (Niewiarowicz et al. 1980),, 30 min. (Knust and Pingel 1992, Knust et al. 1995), 45 min. (Alexieva et al. 1998) after slaughter of birds. The results obtained by Knust and Pingel (1992), Knust et al. (1995) indicated that glycolysis is nearly finished in duck muscles 30 min. after

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slaughter. Therefore we suggested to accept as initial pH values measured 15 min. after slaughter of birds. Referring to the pH_2, all authors agreed to measure it at 24 hours after slaughter.

The values of pH_1 were higher and L^*_1 and b^*_1 were lower than the pH_2, L^*_2 and b^*_2 respectively. However in case of a* parameter no relations could be established as a^*_1 values were for some muscles on the same level, for others higher or lower in comparison with a^*_2 , regardless of kind of duck groups and muscles (see table 1). The colour of all the investigated muscles was defined as pink-red with intensity in interval from 5.4 to 8.1. From the obtained data results that glycolysis runs slightly slower in breast muscles (higher pH_1) than in thigh muscles of investigated birds. The rate of glycolysis in examined duck muscles was smaller than in chicken muscles. Kijowski and Niewiarowicz (1978), found significantly lower values pH_{15} for breast and thigh chicken muscles.

Taking into consideration lack of relevant standards concerning the time of measurement and values of pH_1 in duck muscles, it is very hard to interpret those results. But, the pH_1 suggested that some muscles with the highest pH can be the DFD-like type muscle. On the other hand in general the muscles with higher pH were not characterised by significantly lower L_1 and /or L_2 values. They were not darker and even sometimes were lighter. Beside that the pH_2 values in all investigated muscles were within the interval from 5.71 to 6.06 that has been universally recognised as an indicator of a normal type of poultry meat. On this basis, all of the investigated muscles could be classified as normal type of meat.

Table 1 Colour parameters and pH values of breast and thigh muscles from drakes.

	pH_1	L^*_1	a^*_1	b^*_1	pH_2	L^*_2	a^*_2	b^*_2	SE
Breast									
X (P-33)	6.53	36.6	19.6	1.5	5.90	43.6	19.4	5.7	5.9
SD	0.13	1.10	1.10	0.48	0.13	1.35	0.85	1.14	0.41
X (K-2)	6.48	35.4	19.1	1.4	5.94	42.2	19.4	6.7	6.4
SD	0.034	0.86	0.26	0.47	0.021	0.54	1.23	0.84	0.48
X (A-3)	6.57	35.3	20.9	1.6	5.86	43.0	20.3	5.0	6.7
SD	0.078	1.02	0.45	0.37	0.029	3.53	0.60	0.47	0.79
Thigh									
X (P-33)	6.47	40.7	18.0	3.7	5.96	44.0	19.0	6.2	5.8
SD	0.13	1.22	0.58	0.50	0.12	1.41	1.10	0.41	0.34
X (K-2)	6.31	40.0	16.0	2.8	6.03	41.5	16.7	4.9	6.5
SD	0.16	0.99	0.94	0.57	0.03	0.95	1.08	0.27	0.32
X (A-3)	6.40	41.6	18.1	3.9	5.92	44.1	18.2	6.5	5.8
SD	0.17	1.54	0.73	0.63	0.08	1.42	0.93	0.32	0.30

X- average values, SD- standard deviation, SE- sensory evaluation

The SE data are average assessments given by 8 testers

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The remaining data for breast are average values of two measurements and for thigh only of one measurement.

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CONTENTS

SLOW CHILLING OF TURKEY CARCASSES CAN CAUSE PROTEIN DAMAGE AND PALE, SOFT, EXUDATIVE MEAT

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Keywords : meat quality, PSE, turkey, protein denaturation, chilling

Abstract

To evaluate the relationship of slow chilling to pale, soft, and exudative (PSE) meat, 48 male turkeys were processed and chilled at 0, 10, 20, or 30 C for either 45 or 90 min prior to deboning. Temperature, pH, color, gel strength, cook loss, expressible moisture, and precipitated phosphorylase were determined on the fillets. Through 105 min postmortem (PM), the four temperature treatments produced significantly different breast muscle chilling rates. The 30 C-treated carcasses had a more rapid muscle pH decline, lower ultimate pH, paler color, higher drip and cooking losses, and weaker gel strength than fillets from the carcasses treated at 0 C for shorter periods. Phosphorylase precipitation also increased in samples treated with a slower chilling rate. Achieving a breast muscle temperature of less than 36C by 60 min PM and 28C by 90 min PM may reduce the PSE-like characteristics.

Résumé

Cette étude a été conduite pour évaluer l'effet d'une réfrigération lente de la viande sur l'incidence du caractère PSE (pale, molle et exudative). 48 carcasses de dinde ont placé à une température de 0, 10, 20 ou 30 C pendant 45 ou 90 minutes avant d'êtredésossées. La température, le pH, la couleur, la force mécanique après gélification, les pertes à la cuisson, l'humidité , la solubilité totale des protéines et la dénaturation de la phosphorylase . Les carcasses traitées à 30 C ont un pH qui baisse plus rapidement , un pH ultime inférieur, une couleur plus pâle, une perte de poids, un égouttage supérieur, une force mécanique après gélification inférieure aux filets issus des carcasses placées à 0 °C. La précipitation du phosphorylase est supérieure pour les carcasses placées à 20 et 30°C. Si l'on atteint une température des muscles du filet de

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36°C à 60 minutes (ou 28°C à 90 minutes après l'abattage), on peut réduire les risques de viandes PSE.

Introduction

Postmortem factors such as chilling regimes can influence the development of PSE meat (Honikel and Fischer, 1977; Offer, 1991). Studies in turkeys have indicated that postmortem temperatures of 30 to 37 C accelerated postmortem metabolism (Khan and Frey, 1971). McKee and Sams (1998) also found that holding carcasses at elevated temperatures of 40 C accelerated postmortem glycolysis and resulted in pale meat with increased drip loss and cook loss when compared to carcasses chilled at 0 C. Alvarado and Sams (2002) found that color, water holding capacity, texture, and product integrity are negatively affected in slow chilled turkey carcasses. The objectives of this research were to evaluate the relationships between muscle chilling rates, protein denaturation, and the development of PSE meat.

Material and Methods

A total of 48 turkey toms (22.5 wks) were processed in each of two trials. Feed was withdrawn 12 hours prior to processing, but the birds had access to water until 2 hr prior to conventional processing. Following evisceration, the carcasses were immersion chilled in 0, 10, 20, or 30 for either 45 (60 min PM) or 90 min (105 min PM) prior to deboning. Temperature, pH, L* value, gel strength, cook loss, and phosphorylase were determined. The data was subjected to ANOVA and means were separated using Duncan's multiple range test with a significance level of $P < 0.05$.

Results and discussion

Through 105 min PM, the four temperature treatments produced significantly different breast muscle chilling rates with the higher chilling temperatures resulting in higher breast muscle temperatures. In birds exhibiting PSE meat, there was a rapid pH decline early PM. The carcasses chilled for 45 min at 20 and 30 C and those chilled for 90 min at 30 C had significantly lower pH than those chilled at 0 C (Tables 1, 2). Carcasses with a breast muscle temperature of approximately 36C or higher (45 min chilled) or approximately 28C or higher (90 min chilled) reached an ultimate pH

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earlier PM, indicating a faster rate of rigor development as compared to more rapidly chilled carcasses (Figure 1,2).

At 60 min PM, the carcasses chilled at 30 C had significantly higher L* values indicating lighter fillets than the carcasses chilled at 0 C (Table 1). At 105 min PM, carcasses chilled at 10, 20, and 30 C had significantly higher L* values than those chilled at 0 C (Table 2). The effect of carcass temperatures on L* value indicate that at 60 minutes PM, there was an increase in L* value between 35 and 36 C (Figure 3). At 105 min PM, there was a significant increase in L* values of fillets having a muscle temperature of 28 C or higher indicating lighter fillets (Figure4).

Carcasses chilled in 30 C for 60 minutes had increased cook loss, decreased gel strength, lower ultimate pH, and a higher amount of precipitated phosphorylase compared to the other treatments (Table 1). Since low pH and high carcass temperatures early PM can lead to increased protein precipitation, a decrease in protein solubility will decrease the strength of heat induced gels which can cause sliceability problems in cooked whole muscle products. When chilled for 90 min, the lowest ultimate pH along with an increase in precipitated phosphorylase was achieved in carcasses chilled at 30 C (Table 2). Also, L* value was significantly lower in carcasses chilled at 0 C for 90 minutes compared to the remaining groups and cook loss and gel strength were also decreased.

Therefore, the results of this study indicate that there is a relationship between chilling time and temperature with 30 C chilling temperatures for 45 and 90 min resulting in pale, soft, and exudative meat. Therefore, processors should chill turkey carcasses rapidly early postmortem to prevent poor meat quality problems. Achieving a breast muscle temperature of less than 36C by 60 min PM and 28C by 90 min PM may reduce the PSE-like characteristics.

Table 1. Means of Meat Quality Parameters for Turkey Pectoralis Fillets Deboned at 60 min PM

Temperature (C)					
Parameter[1]	0	10	20	30	Pooled SEM
Temperature C	34.83^{b}	35.39^{b}	36.18^{b}	39.31^{a}	0.43
pH	5.93^{a}	5.89^{ab}	5.86^{b}	5.84^{b}	0.02
pH (24 h)	5.85^{a}	5.85^{a}	5.83^{a}	5.74^{b}	0.02
L* Value	53.75^{b}	55.60^{ab}	54.93^{ab}	56.25^{a}	0.41
Cook Loss (%)	16.87^{b}	18.52^{ab}	17.82^{ab}	21.40^{a}	0.72
Gel Strength (kN)	21.45^{a}	18.38^{a}	19.3^{a}	13.24^{b}	1.12
Phosphorylase[2]	52.36^{b}	55.50^{b}	53.92^{b}	90.27^{a}	0.05

[a,b]Means with no common superscript within each row differ ($P<0.05$)

[1]All parameters measured at deboning except 24 h pH

[2]Pellet (precipitated) Phosphorylase is reported as ng/5ug of protein applied per lane

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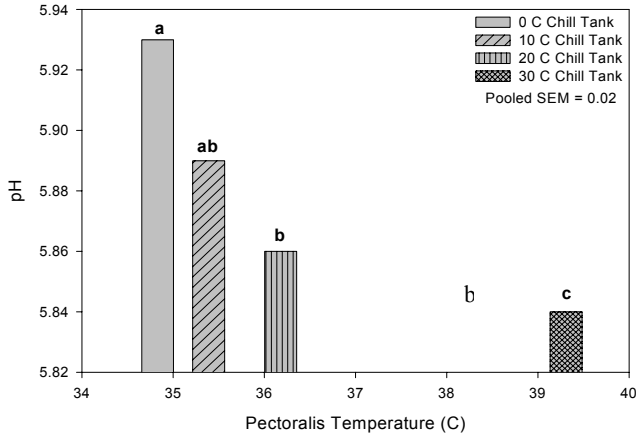
Table 2. Means of Meat Quality Parameters for Turkey Pectoralis Fillets Deboned at 105 min PM

Temperature (C)					
Parameter[1]	0	10	20	30	Pooled SEM
Temperature C	27.28^{c}	28.85^{c}	31.23^{b}	35.66^{a}	0.43
pH	5.83^{a}	5.81^{ab}	5.79^{ab}	5.77^{b}	0.02
pH (24 h)	5.85^{a}	5.81^{ab}	5.78^{b}	5.73^{c}	0.01
L* Value	54.28^{b}	56.39^{a}	56.33^{a}	56.46^{a}	0.36
Cook Loss (%)	20.21^{b}	23.75^{a}	20.82^{b}	22.10^{ab}	0.48
Gel Strength (kN)	20.78^{a}	19.44^{ab}	15.96^{b}	16.67^{ab}	1.03
Phosphorylase [2]	33.90^{b}	36.08^{b}	51.91^{ab}	73.42^{a}	0.05

[a,b]Means with no common superscript within each row differ (P<0.05)
[1]All parameters measured at deboning except 24 h pH
[2]Pellet (precipitated) Phosphorylase is reported as ng/5ug of protein applied per lane

Figure 1. Pectoralis temperatures and postmortem pH decline in carcasses deboned at 60 min postmortem.



[a,b]Means with different superscripts are different (P<0.05)

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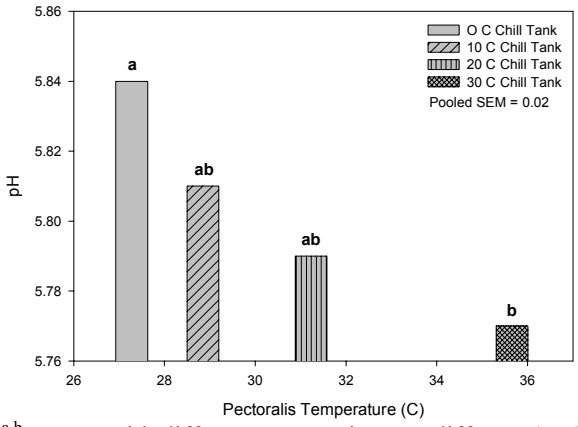
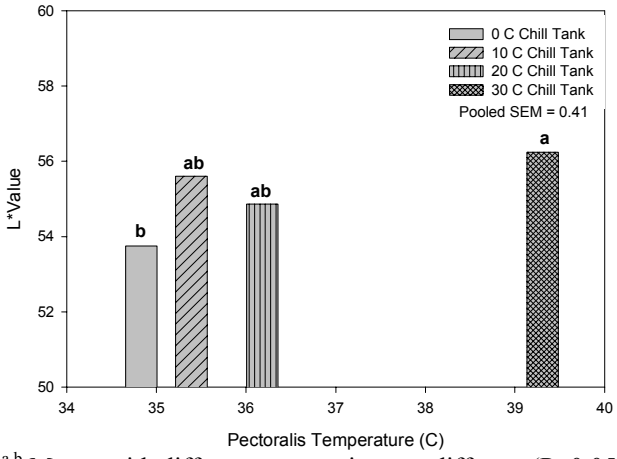
Figure 2. Pectoralis temperatures and postmortem pH decline in carcasses deboned at 105 min postmortem.



[a,b] Means with different superscripts are different (P<0.05)

Figure 3. Pectoralis temperatures and L* values from carcasses deboned at 60 min postmortem



[a,b] Means with different superscripts are different (P<0.05)

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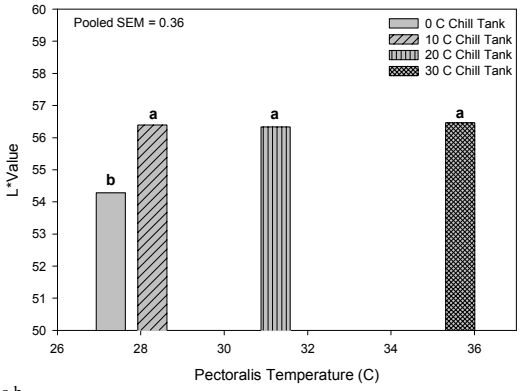
Figure 4. Pectoralis temperatures and L* values from carcasses deboned at 105 min postmortem



[a,b] Means with different superscripts are different (P<0.05)

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COMPARISON OF MEAT TEXTURE IN CHICKEN

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Keywords: mechanical properties, texture, meat, chicken

Abstract

The aim of this study is to compare the mechanical behaviour of meat samples from three lineages of poultry (COU NU, KABIR, and ROSS) obtained under similar procedures. We used compression test with two successive cycles involving a probe of 1 cm² because its corresponds to the action of the masticatory apparatus during chewing of food. Stress-strain curves were obtained and two parameters determined: (i) constraint (N/cm^2) for destructuring the food material and (ii) area under the curve of the first cycle. The analysis of variance showed that the constraint used for fracturing meat from COU NU was significantly smaller than for the other lineages. The surface under the curve toward the stress resulting of meat fracture was also significantly smaller for the COU NU lineage. Chewing work at the first masticatory cycle for COU NU obtained in another study was significantly smaller confirming that sensory evaluation during mastication and dynamic measurements provide similar results in the comparison of three poultry lineages only different by genetics.

Résumé

La résistance mécanique du muscle M. *pectoralis* mesurée à l'aide d'un test de compression à deux cycles en utilisant une sonde plate de 1 cm² de surface a été déterminée dans le cas de trois races de poulets (COU NU, KABIR et ROSS) élevés et abattus, à 14 semaines, dans les mêmes conditions. Les échantillons de viande étaient maintenus entre deux parois fixes afin de limiter leur déformation dans une direction, parallèle aux fibres musculaires. Deux variables ont été déterminées pour chaque courbe contrainte-déformation : (i) la contrainte à la rupture et (ii) le travail nécessaire pour atteindre cette rupture. La contrainte nécessaire pour provoquer la rupture des

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échantillons était significativement plus faible pour le poulet COU NU. Il en était de même pour le travail nécessaire pour atteindre cette rupture. Ces résultats peuvent être reliés à ceux obtenus dans une autre étude montrant que le travail du premier cycle de mastication chez des sujets humains mâles et femelles est aussi significativement plus faible dans le cas du poulet COU NU..

Introduction

Four principal quality factors are recognized for food: appearance, flavour, texture and nutrition (Bourne, 2002). Bourne (2002) defines the textural properties of the food as a group of physical characteristics arising from the structural elements of the food. In meat, these structural elements are complex and produce a composite material mainly involving fibers surrounded by connective tissue, water and lipids (Tornberg, 1996). These elements, particularly muscular fibers containing the contractile proteins are orientated into the muscle resulting in an anisotropy of this food material that produces a complex mechanical behaviour (Lepetit and Culioli, 1994).

A large number of recent studies analyze the mechanical response of the meat from different productions in relationship with the sensory assessments (Mathevon et al., 1995; Mioche et al., 2002) to understand the association between tenderness and hardness perception, masticatory sensory-motor control and chewing efficiency that influence the whole digestive process. Force-deformation relationship during biting or chewing of meat is directly linked to the texture properties that can be described with various mechanical tests (Honikel, 1998; Peyron et al. 1994). This relationship relates to a compression of the food between the teeth cuspids. Mathevon et al. (1995) correlated some electromyographic characteristics of masticatory muscles and data obtained from dynamic measurements using compression and shear tests. These authors showed that assessment of hardness was correlated to destructive shear or non destructive compression tests of cooked meat of beef. These results are rather contradictory to others (Martens et al., 1982; Tornberg and Persson, 1988). Cooking temperature clearly influences the mechanical properties of the meat used to determine its tenderness or hardness because the structural elements are modified under the process of heating.

A major insight into understand the relationship from structure to texture (Wilkinson et al., 2000) for explaining the selection by consumers is to obtain comparative mechanical data in meat obtained from animals either with different genetics and produced under similar technological procedures (i.e., husbandry, food, density) or with similar genetic but produced under various procedures. In such comprehensive approach quantitative analysis of texture of meat poultry are relatively scarce.

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The aim of this study is to compare the mechanical behaviour of meat samples issued of three lineages of poultry obtained under similar procedures. We used compression test in our analysis as this test corresponds to similar action of the masticatory apparatus during chewing of food. These data are finally compared with sensory-motor evaluation obtained for the three poultry lineages by a panel of young adult (male and female) consumers.

Material and methods

Compression testing was done using a Lloyd Instruments (Model TA500), and data collected with the software Nexigen II (Fig. 1). All the date were measured on breast muscle (M. pectoralis) of animals slaughtered at 14 weeks. All production conditions (e.g., density, food, temperature) were similar for the three poultry lineages as they were bred in the same building at the Farm of the C.A.R.A.H.

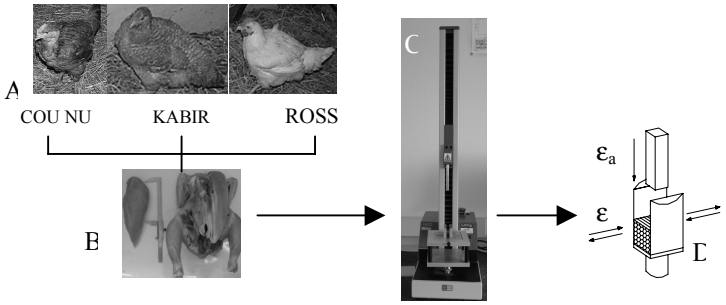

Fig 1. Experimental setting. Meat samples from three poultry lineages (A) were slaughtered in similar conditions and the M. pectoralis cut of the carcasses (B). Samples of 1 cm^3 were tested using a compressive probe (C) in longitudinal (D) configuration. ε_a : uniaxial stress ; ε : free strain (modified from Lepetit and Culioli, 1994).

Ten raw meat samples for three specimens of each lineage underwent two successive compression cycles at a speed of 1mm/s up to 70 % deformation corresponding to destructive tests in all samples. The probe was orientated perpendicular to the fiber axis of the samples placed in a cell with two lateral walls to limit the free strain in only one direction parallel to the muscular fibers (Fig. 1). Two parameters were determined on the stress-strain curves in this preliminary study: (i) constraint (N/cm^2) for destructuring the food material (maximum stress) and (ii) area under the curve of the first cycle toward the fracture point. All these data were statistically analysed using the Statgraphics version 5.0 plus (Manugistics, Inc, 2000). Shapiro-Wilk's W-statistic for Normal distribution of the data and F_{max} –test for equality of variances assumed that values can be used for ANOVA. In this study, we used a two-level nested ANOVA to

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test the significant difference in variables between the poultry lineages and among samples within poultry lineages. F-statistics for the fixed effect of poultry lineages were obtained by dividing the mean square of the poultry by the mean square for the random effect (sample). The effect of sample within poultry lineages was then tested over the residual. Student Newman-Keuls tests were used for comparing the differences of the means.
Histological results were obtained from transverse sections of samples of the muscle at 7µm using the standard methods for staining with Trichrom Masson (Bancroft and Stevens,1996).

Results and discussion

Figure 2 shows a typical view of the different levels of the organisation of the muscle M. pectoralis at 14 weeks. Briefly, the mass of fibers are arranged in regular polygonal bundles surrounded by connective tissue. Preliminary analysis did not show any significant differences in structural properties of the muscle in the three lineages.

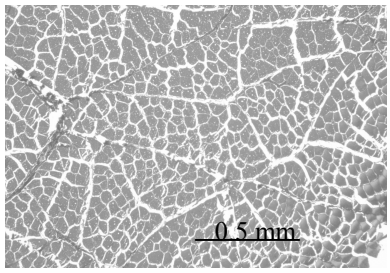

Fig 2. Transverse section of the M. pectoralis in COU NU

Typical stress-strain curve (Fig. 3) is divided into two successive phases before fracturing the meat sample. First, the force and deformation increases steadily showing a short period of elastic response. Second, a plastic deformation including various numbers of high modification of the muscular structures was observed. This curve corresponds to fracture of type 3 (Bourne, 2002) with steps corresponding to successive crack propagation within the material An initial cracking can occur before the definite fracture. From these curves, fracture into the muscle samples occurred before maximal deformation (70%) confirming that the test allowed to measure the stress needed for destructuring the meat material. In this analysis, we did only compare the values corresponding to definite fracturing of the meat samples.
The analysis of variance shows a significant differences for the stress needed for destructuring the meat from the three lineages (Nested-ANOVA, d.f.=2, 8; F=4.46,

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P<0.05). The constraint used for fracturing meat from COU NU was significantly smaller than for the other lineages (Fig. 4). The surface under the curve toward the stress resulting in meat fracture was also significantly smaller for the COU NU lineage (Nested-ANOVA, d.f., 2, 8; F=8.96; P<0.05). The mean stress for COU NU was 0.020 ± 0.0017 N/cm² compared to the mean for KABIR (0.033 ± 0.012 N/cm²) and ROSS (0.055 ± 0.0016 N/cm²).

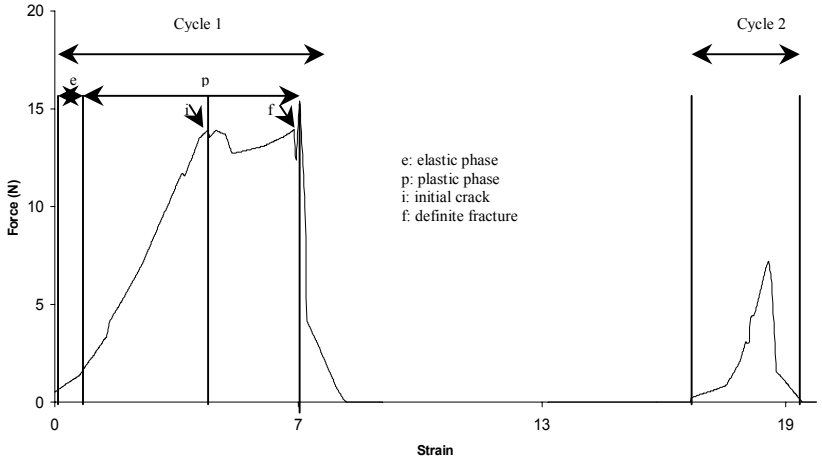

Fig 3. Typical stress-strain curve obtained for a meat sample of KABIR poultry lineage.

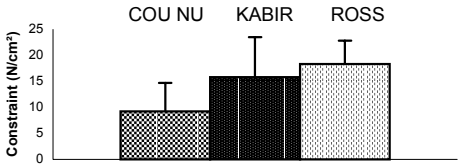

Fig 4. Comparsion between the mean of stress needed for fracturing the three poultry lineages the M. pectoralis in COU NU

Our results show that the dynamic measurements asses that meat of COU NU has the smaller hardness (*sensu* Bourne, 2002). This result confirms that sensory evaluation of meat texture triggered at the earlier contact (first chew) between the teeth (molars) and the meat during compression between the jaws (Bels et al., this 2003). Chewing work at the first masticatory cycle for COU NU is significantly smaller than for both other lineages confirming that sensory evaluation during mastication and dynamic measurements provide similar results in the comparison of three poultry lineages only different by genetics.

In conclusion, this preliminary analysis shows possible correlation between psycho-physiological experiments based on EMG recording obtained for meat samples from

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different poultry lineages cooked in same conditions (92°C) with mechanical properties of the crude meat. In our analysis, we did not considered the effect of temperature on the fluctuation of sensory evaluation and mechanical measurement as demonstrated in previous studies (Martens et al., 1982; Mathevon et al., 1994).

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CHICKEN MUSCLE HOMOGENATE DENATURATION AND GELATION PROPERTIES: EFFECT OF PH AND MUSCLE FIBER TYPE

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Keywords : chicken; muscle homogenate; denaturation; gelation; pH

Abstract

Denaturation and gelation properties (DSC and TMA determination) of chicken breast and thigh muscle homogenates (4.5% protein, pH 6.00 and 6.30) were compared to determine the influence of muscle fibre types. Breast muscle homogenate gels were weaker at pH 6.00 than thigh muscle homogenate gels and the reverse relation was found at pH 6.30. Enthalpies for breast and thigh muscle homogenates at pH 6.00 and 6.30, respectively, were not significantly different. Breast and thigh muscle homogenates underwent three (at pH 6.00) and four (at pH 6.30) transition temperatures. Breast muscle homogenate transition temperatures at pH 6.00 were higher than for thigh muscle homogenates and they were comparable at pH 6.30. Therefore, variations in the thermal unfolding process only partly explain the differences in gel properties of muscle homogenates from white and red chicken muscles.

Résumé

On a comparé les qualités de dénaturation et de gélification (par DSC et TMA) d'homogénats de muscles du filet et de la cuisse de poulets (4.5% de protéine, pH 6.00 et 6.30) dans le but d'évaluer l'effet du type de fibres sur ces qualités.. A pH 6.00, les gels réalisés à partir des muscles du filet était plus fragiles que ceux réalisés à partir des muscles de la cuisse. L'inverse était observé à pH 6.30. Les enthalpies des homogénats de muscles du filet et de la cuisse à pH 6.00 et 6.30 respectivement n'étaient pas significativement différentes. Les homogénats des muscles de la cuisse et du filet était caractérisés par trois températures de transition à pH 6.00 et quatre à pH 6.30. . A pH 6.00 les températures de transition des homogénats de muscles du filet étaient plus élevées que celles des homogénats des muscles de la cuisse. A pH 6.30, leurs valeurs

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étaient comparables. En conclusion, les variations de cinétiques de dénaturation ne peuvent expliquer qu'en partie les différences de gélification observées entre les muscles blancs (filet) et rouges (cuisse) de poulet.

Introduction

Gelation properties of myofibrillar proteins are influenced by the distribution of specific fibre types in muscle samples from which myofibrillar proteins are extracted [Xiong, 1994; Wang and Smith, 1994; Lefevre et al., 1999] as well as by protein concentration and pH [Lesiów and Xiong, 2001a]. The latest literature concerning denaturation of poultry muscle myofibrillar proteins stressed that under dynamic conditions aggregation plays a major role in producing gel elasticity differences between white and red myofibrillar proteins [Lesiów and Xiong, 2001b].
The objective of this study was to compare denaturation and gelation properties of chicken breast and thigh muscle homogenates at an intermediate protein concentration (4.5%) and pH 6.0 and 6.3.

Materials and Methods

Investigation was performed on chicken broilers stored at 2-4^{o}C after 24 hr *post mortem*. The combined breast or thigh muscle from two broiler carcasses were separately ground through a 3 mm plate. The pH was measured with a pH-meter and electrode inserted into the ground meat or muscle homogenates.
Meat homogenates were obtained by homogenisation of 15 g ground breast (B) and 17.67 g ground thigh (T) muscles with 60 ml 0.67 M NaCl cold solution (at pHs 6.50 and 6.00 for breast muscle, and 6.10 and 5.40 for thigh muscle) for 1 min at 4000 rpm. The final pH of homogenates, if necessary was re-adjusted with 0.1 N HCl or 0.1 N NaOH to 6.30 and 6.00. The protein content in meat homogenates was 45.5 mg/g.
For each pH value, two parallel samples from meat homogenate were placed in the glass tubes (25 x 40 mm) and were heated isothermally at 70^{o}C for 30 min in a water bath. After heating, gels were cooled in an ice slurry for one hour and stored overnight at 2-4^{o}C. Deformation of the gels upon compression was measured on a modified "Höppler" Rheo-Viskometer equipped with a flat 14.8 mm diameter plunger. Stepwise changed stress, being the multiple of 0.285 kPa, was measured after each 30 s. The stress at failure was used to express the gel strength (kPa).
Nondestructive, oscillatory measurements of the muscle homogenates during gelation (27-90^{o}C, 3^{o}C/min) were performed using a TMA/SS 150U (Seiko). Rheological

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properties were described in terms of transition temperatures (T'_{max} and T'_{min}) and storage modulus at peak (G'_{max}) and at the end of heating (G'_{74^oC}).
Transition temperatures and enthalpy of denaturation for muscle homogenates (30-95^{o}C, 3^{o}C/min) were performed using a DSC 22C (Seiko).
Within each of three to five replications, for each pH value and muscle type, two parallel measurements for gelation properties and denaturation transition temperatures (T_m) were made. The test "Statistica" was used to identify significant differences at $P<0.05$.

Results and discussion

The gel strength of breast muscle homogenates increased with increasing pH from 6.0 to 6.30 (Table 1) [Lesiów and Xiong 2003]. The strength of thigh muscle homogenate gels at pH 6.00 was not significantly different from corresponding value at 6.30. The gel strength of thigh muscle homogenates was significantly higher than that of breast muscle homogenates at pH 6.00. It is in accordance with the observation of Lan et al. [1995] that showed chicken breast myofibrils produced weaker gels than thigh myofibrils at 5% protein concentration and pH 6.00. By contrast, gel strength of breast muscle homogenates was significantly higher than thigh muscle homogenates at pH 6.30 (by 14.63%). Xiong [1992] found that chicken leg myofibril gels formed at pH 5.50-5.80 were more rigid than breast gels, and above pH 6.00, this was reversed. It was proven in another study [Xiong and Blanchard, 1994] in which myofibrillar proteins isolated from chicken breast muscle formed stronger gels than from thigh and drumstick muscles at pH from 5.87 to 6.53. The finding was consistent with the results obtained for muscle homogenates even though the pH at which breast muscle homogenate gels were stronger than thigh gels was shifted to higher pH value, i.e., pH 6.30.
The values of G'_{max} and G'_{75^oC} (determined by thermomechanical analysis TMA) for breast meat homogenate at pH 6.30 were higher than that at pH 6.00, and for thigh meat homogenate these corresponding values were not significantly different (Table 1). At pH 6.00 storage modulus at peak (G'_{max}) and at the end of heating (G'_{75^oC}) of breast muscle homogenate was lower than for thigh muscle homogenate. However, at pH 6.30 a reverse trend was observed only for storage modulus at peak (G'_{max}) with breast muscle homogenate producing a more elastic gel.
Chicken breast SSP (Salt Soluble Protein) and myofibrils developed a more elastic gel structure (greater G'_{max} and G'_{74^oC}) than thigh protein at pH 6.00 and 5.87-6.38 [Xiong 94; Xiong and Blanchard, 1994], indicating that thigh proteins were less able to form a cross-linked gel network. However, differing from muscle homogenate systems, a pH 5.50-6.50 and 5.87 favours the formation of an elastic gel network ($G'_{80 \text{ or } 74^oC}$) for isolated breast muscle proteins when compared to other pH conditions [Wang et. al., 1990; Xiong and Blanchard, 1994]. Moreover, the thigh muscle myofibrils formed also more elastic gels

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(G'_{74^oC}) at pH 5.87 than at 6.38 while in the present study changes of G'_{max} and G'_{75^oC} for thigh muscle homogenates at pH 6.00 and 6.30 were insignificant.

Breast and thigh muscle homogenate had higher calorimetric enthalpies (DSC analysis) at pH 6.0 than at pH 6.30 (Table 1). Breast muscle homogenate enthalpy at pH 6.0 was lower and at pH 6.30 was higher than thigh muscle homogenate enthalpy, respectively but the values were not significantly different. Breast and thigh muscle homogenates underwent three transition temperatures at pH 6.00 and four transition temperatures at pH 6.30. Transition temperatures of breast muscle homogenate at pH 6.0 were higher than at pH 6.30 and for thigh muscle homogenate the first and the second transition temperatures were comparable while the third transition temperature was higher at pH 6.0 than at pH 6.30. Breast and thigh muscle homogenate transition temperatures at pH 6.00 and 6.30 were comparable.

Xiong and Brekke [1990a] reported that hen breast SSP (30-35 mg protein/ml, 0.1 M NaCl, 10^{o}C/min) at pH 6.0 and 6.5, and leg SSP at pH 6.0 and 6.5, showed a single thermal transition which ranged from 55 to 64^{o}C (60.9 and 63.8^{o}C for breast SSP, and 59.9 and 62.0^{o}C for leg SSP, respectively). The higher pH of breast and leg SSP resulted in higher temperature of first thermal transition which was reverse for breast and thigh muscle homogenate. Xiong et al. [1987] also found that DSC thermograms of myofibrils (10-12 mg/ml, 0.1M KCl, 10^{o}C/min, pH 7.1-7.2) from chicken breast muscle yielded transitions at 52.8, 60.8 and 68.7^{o}C, whereas thigh gave two transitions at 60.3 and 69.5^{o}C. Discrepancies in DSC between SSP or myofibrils and muscle homogenates can be attributed to the animal differences (hen-chicken) and variations in protein concentration, pH, ionic strength and complicate the comparison of the specific differences between chicken muscle proteins and homogenates.

Dudziak and Foegeding [1988] did not detect significant differences in onset denaturation temperature (49.8 and 50.5^{o}C), maximal denaturation temperature (59.0 and 59.5^{o}C) and enthalpy of denaturation (2.08 and 2.26 cal/g) of myosin and actomyosin mixtures (40mg/ml, 1^{o}C/min) isolated from white and red turkey muscles. Likewise, Xiong and Brekke (1990a,b) did not find pronounced differences in onset temperature for protein unfolding (32 or 30^{o}C), in the maximal transition temperature (60.9 and 59.9^{o}C), and in hydrophobicity (a measure of the extent of exposing hydrophobic regions), during heating hen breast and leg SSP. Hence, the rate and extent of protein unfolding or conformational changes appeared similar for breast and leg SSP. However, above cited authors observed a great disparity in gelation properties between proteins from white and red muscles. Therefore, according to Xiong (1994) variations in the thermal unfolding process were not directly responsible for the differences in gel properties of myofibrillar proteins from white and red poultry muscles.

Variations in the thermal unfolding process only partly explain the differences in gel properties of muscle homogenates what could be confirmed with following facts:

- higher gelation properties of breast muscle homogenate and lower transition temperatures at pH 6.30 than at pH 6.00;

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- insignificant changes in gelation properties of thigh muscle homogenates and first and second transition temperatures at pH 6.00 and pH 6.30;
- lower gelation properties of breast than thigh muscle homogenates at pH 6.00 corresponding with higher transition temperature for breast than thigh muscle homogenate;
- better gelation properties of breast than thigh muscle homogenates at pH 6.30 occurring at comparable transition temperatures for both muscle homogenates.

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Table 1. Stress at failure (Sf); transition temperatures (T'_{max} and T'_{min}); storage modulus at peak (G'_{max}) and at the end of heating (G'_{74^oC}); DSC transition temperatures (T_m) and enthalpy of chicken breast and thigh muscle homogenate at different pH.

Muscle	Breast		Thigh	
Attribute	$pH_{6.0}$	$pH_{6.3}$	$pH_{6.0}$	$pH_{6.3}$
Sf (kPa)	5.16 (0.72)^a	7.80 (0.53)^b	7.27 (0.39)^{bc}	6.66 (0.53)^c
T'_{max} (^{o}C)	52.70 (1.16)^a	55.63 (0.96)^b	52.47 (0.74)^a	54.78 (0.93)^b
T'_{min} (^{o}C)	58.56 (0.20)^b	59.03 (0.95)^b	55.73 (0.53)^a	58.46 (0.91)^b
G'_{max} (kPa)	5.96 (1.42)^a	71.38 (3.43)^b	47.34 (7.09)^c	47.39 (9.16)^c
G'_{74^oC} (kPa)	72.44 (8.52)^a	163.4 (2.84)^b	144.8 (12.62)^c	152.5 (2.33)^{bc}
T_{m1} (^{o}C)	55.26 (5.01)^b	50.18 (2.82)^a	51.79 (0.13)^{ab}	49.72 (1.49)^a
T_{m2} (^{o}C)	64.66 (1.97)^b	56.46 (3.64)^a	61.08 (0.65)^{ab}	58.49 (3.93)^a
T_{m3} (^{o}C)	73.28 (5.00)^b	64.43 (2.91)^a	71.57 (3.11)^b	62.36 (2.89)^a
T_{m4} (^{o}C)		74.52 (3.44)^a		73.06 (3.89)^a
Enthalpy (J/g)	2.00 (0.60)^{bc}	1.52 (0.43)^{ab}	2.50 (0.10)^c	1.27 (0.36)^a

Means with different superscript in the same row are significantly different at $P<0.05$. Values in parenthesis are standard errors for means.

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HISTOMORPHOLOGIC AND STEREOLOGIC ANALYSIS OF TISSUE COMPONENTS WITHIN THE CHICKEN MEAT PRODUCTS

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Keywords : Chicken meat, histomorphological analysis, stereologic analysis, sausages

Abstract

The investigations were carried out on medium lasting sausages produced by "Koka" meat precessing industry in Varaždin. The first type of these sausages was made from boneless chicken meat by adding red pepper, button mushrooms and cheese; the second type was made from smoke-dried chicken breasts; and the third one was a boiled sausage of frankfurter type. The share of several tissue components (muscular, connective and fat tissue) was evaluated by means of histologic and stereologic procedures. As the investigations did not detect the presence of osseous and cartilaginous tissue, it indicates that the process of bone elimination was well done. Concerning the share of tissue components taken from the extremities and from the middle of sausage samples, no essential difference was noted whatsoever, which is a proof of a good technologic process of mixturing of meat mass and additives. In all investigated products was found a bigger percentage of muscular than of connective and fat tissue. So it can be stated that the investigated products were of a good quality.

Résumé

L'étude porte sur des produits de charcuterie cuite, type saucisses, produites par la société "Koka" implantée à Varaždin. Le premier type de produit est élaboré à partir de viande de poulet (sans os), de poivre rouge, de champignons et de fromage : Le deuxième produit est composé de filets de poulets fumés-séchés et le troisième est de type saucisse de Francfort. La répartition des différents constituants (tissu musculaire, conjonctif et adipeux) a été évaluée à l'aide de techniques histologiques et stéréoscopiques. Aucune d'entre elles n'a révélée la présence de résidus osseux ou cartilagineux ce qui suggère une bonne efficacité du processus d'élimination des os.

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Au niveau de la répartition spatiale des composants tissulaires, aucunes différences notables n'ont été relevées au niveau des extrémités ou au milieu du produit. Ceci confirme une bonne maîtrise du process de mélange de la viande et des additifs. Dans tous les produits, la proportion de tissu musculaire est largement supérieure à celles des tissus conjonctifs ou adipeux. En conclusion, on peut retenir que les produits étudiés sont de bonne qualité.

Introduction

An accurate view of the composition of meat products is possible by applying some methods of organoleptic and chemical analysis. A combination of histologic and stereologic investigation methods may give objective results in order to evaluate the proportion of tissue components within meat products. Therefore the histology itself and its methods have an important role in the meat product technology, especially in an objective evaluation of the quality of its contents, but also in order to detect those meat products that may be alterated by organs and tissues unpropiate for such use. In our investigations histologic and histochemical methods were meant to get objective results concerning the tissue and other components within some boiled and medium lasting sausages made from chicken meat. In order to obtain a percentage proportion of each component, a stereologic analysis was applied (Kališnik, 1985). As a measuring method, it completes the view of the quality of meat products by determining the tissue proportion within a volumic unit of any histologic preparation.

Material and methods

The investigations were carried out on the products proceeding from "Koka" meat processing industry in Varaždin: (a) medium lasting sausages made from boneless chicken meat declared containing red pepper, button mushrooms and cheese; (b) medium lasting sausages made from smoke-dried chicken breasts; (c) boiled sausages of frankfurter type.
Ten items of each product were used for the investigation, the samples being taken from both extremities and from the middle of each item in order to obtain an accurate view of the sausage composition and its filler.
For the analysis of morphohistologic construction some samples were fixed in 10% formalin, mounted in paraffin and cut on cryostat into 10 μ thick slices that were stained with hematoxylin and eosin (Romeis, 1968). Other samples were frozen on cryocut and cut into 10 μm thick slices that were stained for the appearance of fats

according to the Oil red method (Romeis, 1968), as well as for the appearance of the connective tissue by the Van Gieson method (Romeis, 1968).
On preparations stained according the mentioned methods, stereologic investigations were performed by means of the projection screen of a Wild M 20 microscope with a 60 x magnification, being applied the multipurpose testing system (Weibel et al. 1966) and the Kališnik (1985) method. In each preparation 10 fields of sight were accounted.

Results

The morphologic construction was investigated in samples from the sausages stained with hemalun and eosin. It is evident that big or minor bundles of skeleton muscular tissue dominate in the preparations made from medium lasting sausages. The participation of muscular tissue is bigger in medium lasting sausages made from smoke-dried chicken breasts. Besides the muscular tissue, connective and fat tissue was noted as well, its share being considerably smaller. Within the boiled sausages of frankfurter type, according to our estimation, the share of connective tissue is also rather big.
Applying the staining method for the appearance of fats some bigger or smaller droplets of neutral fat were noted. Its quantity was bigger in medium lasting sausages with added red pepper, button mushrooms and cheese, and in frankfurter sausages, while it was considerably smaller, i.e. insignificant, in products made from smoke-dried chicken breasts.
Specific staining for the appearance of connective and muscular tissue showed that the quantity of connective tissue is rather small in comparison with muscular tissue, although few connective tissue stained red by this method could be noted within the preparations made from medium lasting sausages with button mushrooms. On the other hand, muscular tissue was stained yellow. Minimal quantity of connective tissue was noted in medium lasting sausages made from smoke-dried chicken breasts, while it was bigger in other types of the investigated sausages.
In slices of each sausage type, besides muscular, connective and fat tissue, one could also notice parts of chicken skins, their share being indicated in specific producer's declaration, which is equally valid as to the quantity of added red pepper, button mushrooms and cheese.
Considering the proportion of tissue components taken from the extremities and from the middle of sausage samples, no important difference was noted.
The results quoted above were obtained on the basis of a subjective estimation. In order to get more objective indicators, some stereologic investigations were made as well. The results are shown in Table 1.

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Discussion

Our findings show that the quantity of muscular, connective and fat tissue in the investigated sausages vary, which depending on sausage type and technological process applied in the production.

The quantity of muscular tissue is bigger in medium lasting sausages made from smoke-dried chicken breasts than in other sausage types. As the product quality depends on the quantity of muscular tissue, it means that the medium lasting sausages made from smoke-dried chicken breasts are a very good product because they contain a lot of muscular tissue and very few fat and connective tissue. We consider that, according to their tissue components, the other sausages are also of a pretty good quality, despite the bigger of connective tissue in medium lasting sausages made with added button mushrooms.

In these investigations there was no essential difference between the tissue components in samples taken from the extremities or the middle of the sausages. This confirms a good technological procedure for miwing meat mass and additives.

One can state that, by combining histologic and stereologic methods, it is possible to get an accurate insight into the composition of sausages. Considering the share of each tissue component it is also possible to form a general impression of the quality of such product (Jelić et al., 1994; Peševski et al., 1998; Nejedli et al., 1998; Nejedli et al., 2001). Therefore such investigations may indicate to producers some defects occurring during the processi such as an excessive adding of fats or connective tissue. The dispersion of fat droplets within the sausage stuffing may be shown by means of the histologic technique. An even dispersion of fat droplets confirms a good mixturing (Heath et al., 1971). As no presence of osseous and cartilaginous tissue was noted, it may indicate that the process of bone elimination was done very well indeed.We consider that these investigations would be welcome in practice because the applied methods are objective and accessible.

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Table 1. Tissue composition of different sausages.

Product		Muscular tissue %	Connective tissue %	Fat tissue %	Empty space %
Delko paprika	Extremity	47,57	9,05	15,64	27,78
	Middle	36,22	6,58	19,75	37,45
	Total	41,89	7,81	17,69	32,61
Smoke-dried chicken breasts	Extremity	60,70	3,29	2,88	33,13
	Middle	68,72	2,88	2,47	25,93
	Total	64,71	3,08	2,67	29,53
Slice with cheese	Extremity	42,81	6,58	18,52	32,09
	Middle	33,12	6,58	27,58	32,72
	Total	37,96	6,58	23,05	32,40
Delko with button mushrooms	Extremity	32,51	7,41	25,51	34,57
	Middle	37,04	6,58	18,93	37,45
	Total	34,77	13,99	22,22	36,01
Chicken frankfurter 100 g	Extremity	41,55	4,12	21,40	32,93
	Middle	37,65	4,53	20,99	36,83
	Total	39,60	4,32	21,19	34,88
Chicken frankfurter 300 g	Extremity	27,77	8,23	20,58	43,32
	Middle	31,68	9,88	21,40	37,04
	Total	29,72	9,05	20,99	40,23

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PRODUCTION OF BIOLOGICALLY ACTIVE INGREDIENTS AND FUNCTIONAL PRODUCTS ON THE BASIS OF RATIONAL USE OF CHICKEN BODIES CUTTING

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Keywords : poultry meat and products of processing, biologically active ingredients, functional products

Abstract

Increasing volumes of poultry processing arise the necessity of developing new rational schemes of poultry cutting under conditions of maximum involving of all available resources into production of varios products. New approaches and principles are based on the use of biocatalitic processes and deep profound knowledge of physico-chemical properties of the raw material. This is especially actual at the processing of feather, skin its derivatives, heads and legs of poultry with the aim of production of new biologically active ingredients and functional additives.

Resume

In the most developed countries the share of poultry products production is 80-90 %. According ti the experts forecasting world meat production will increase from 196 m tons in 1993/95 up to 266 m tons in 2005, the poultryof meat being 83,7 %, with the annual increase 5,6 %. In developing countries per capita meat consumption will increase two times and will be 10 kg [1,2,3]. Simultaneously there will increase the volumes of low valuable and secondary products which neeed the development of new approaches of their rational use on the basis of application of specific ferments and the analysis of protein, lipid, vitamin and mineral content.

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Materials and methods

The object of investigation was the main and secondary raw material (chicken neck skin, stomachs, feather, chicken fat from cock combs, muscule stomach, heads, legs, etc.) of poultry processing (chicken, broiler chickens) produced by "Zabrodnenskaya" poultry plant (Kalach, Voronezh region, Russia), fermental preparations of proteases from hepatopancreace of a king crab and microbial - megaterin G10x from Bacillus megaterium, protofradin G10x from Strepomyces fradia. Common methods of study: mass share of moisture, fat (by Soxlet method and refractometrically), protein «total» (photometrically), ash (gravimetrically); fractional composition of proteins by successive extraction of protein fraction by specific solvents and quantitative determination; fatty and composition of lipids by the method of gas-liquid chromatography at the chromatograph "Varian-3400"; amino acid composition of raw materials and food additives by the method of ionexchange chromatography at the automatic amino acid analyses AAA-T339 (Chehia); attackability of the proteins of the raw material and half-finished products by digestive ferments according to Pocrovsky and Ertanov; emulsifying ability, emulsion stability (by weighing method); water holding capacity (WBC), fat holding capacity (FHC) – in accordance.

Results and discussions

Ferment technologies seemed to be rather attractive in connection with the possibility of organization harmless production with a high degree of receiving the desired result on the production of aminoacids and peptides with minimum racemation. Ferment proteases preparations from hepatopancreace of a king crab and Bacillus megaterium have a high similarity with the connective tissue proteins of poultry meat and by – products and the preparation from Streptomyces fradia splits actively feather keratine. Changing leads to the possibility of regulation protein destruction degree including those, which are hardly digested, and ratio of peptides and amino acids in reacting blend. This allows to obtain products with different functionality due to the aimed dosage of proteins of a connective tissue, to change the degree of digestibility and the rate of products assimilation in a gastrointestinal tract, to guarantee the necessary level of technological functionally of digestive systems.

Biocatalytic processes are used at the treatment of secondary products on the basis of recommended schemes of chicken cutting (fig. 1).

Poultry meat is a high-quality product rich in proteins and which has low caloricity in comparison with pork and beef. Muscle tissue of a chicken contains 18-22 % of protein which have all essential amino acids. Fat content in poultry meat is minimal. Mainly fat is under

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the skin (25 %) and in inter muscle connective tissue (3 %)/ Mass share of pfospholipids in fat of breast muscle – 48 %, leg – 21 %. The share of polynonsaturated fatty acids prevail in composition of fatty acids. Muscule tissue is rich in minerals, water-soluble vitamins, ferments, extractives (up to 1 % - glycogen, lactic acid, glucose).

Meat of mechanical boning of poultry is also characterized by a high food and biological value. It has a higher share of fat and phospholipid compounds, more vitamins (especially of A, E, Д group), the share of calcium is 4 times higher in comparison with meat of hand boning (0,016-0,024 %).

Secondary by-products (muscular stomach, liver, heart) are characterized by a high share of proteins (up to 20 %) with prevailing of water- and salt-soluble fractions and this is near to indices of a muscle tissue on the account of a considerable share of taste – and flavourforming amino acids. A small share of methionine and tryptophane testifies to the expediency of usage of by – products for food, aims in combination with other proteins rich in essential amino acids. These secondary products are enriched with polynonsaturated fatty acids and vitamins.

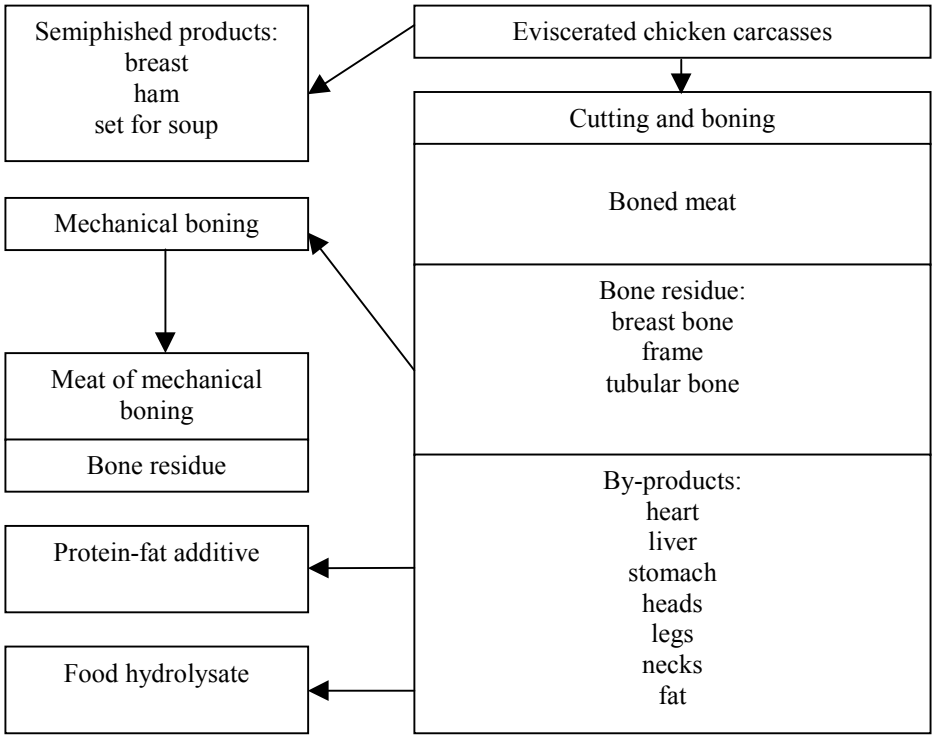

fig. 1

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Chicken skin has a practical interest due to the possibility of enrichment products by proteins – collagen (food fibre), easily digestive and well emulsified fat. It can be used in meat formulations.

Heads and legs can be used as sets for soup or set for meat jelly. They do not have low popularity among consumer but still they contein rather large share of proteins. To use rationally such secondary products it is necessary to apply additional technological methods correcting structural and mechanical properties, and increasing biological value.

In order to destruct strengthened protein structures of a connective tissue it expedient to use ferment preparations, the complexes of which are characterized by high levels of specific proteolytic activity.

Optimization of conditions of a ferment hydrolysis of treated preliminary crushed heads and legs under the influence of above mentioned ferment preparations allow to increase mass share of a soluble protein, to obtain digestible forms of minerals especially calcium and phosphorus, to use connective tissue proteins, to divider fat and protein fractions, and consequently to guarantee giver chemical composition and properties of a product.

Thus, the suggested scheme of cutting and treatment of poultry products includes production of boned meat (output 38,9 % to the mass of initial raw material) – «white» and «red». Its application in formulations of semi-finished products, sausages (stuffed and minced ham) allow to wider considerably the assortment of products from chicken meat how valuable parts, the output of which from mass of eviscerated carcasses is (% from mass): frame – 23,9; breast bone – 7,1; tubular bone – 10,7; necks without skin – 2,1 – are sent to mechanical boning and also for production of different culinary products (sauces-pastes, meat jellies etc).

The scheme of cutting suggests a wide use of secondary products. We developed the production technology of a food protein – fat additive from a skin of land birds as a substitute of main raw materials at the production of minced meat products (meat loaves, stuffed sausages, pastes and etc on).

The addition of chicken broth into protein – fat additive improves its reological properties and guarantec good forming indices. Chilled product is monolithic and has a good form.

The broth contains water soluble substances, valuable proteins (polypeptides, free amino acids, water soluble vitamins) increasing food value of a product.

The use of a chicken broth and melted chicken fat contributes to balancing of protein – fat ratio (1:1) in a half – finished product.

Identification of amino acid composition of food additive showed that it is characterized by presence of all essential amino acids excluding tryptophan. Amino acid scor is from 80 up to 89 % (isoleucine, threonine and phenilalanine). As for non-essential, amino acids there prevail glutaminic and asparaginic acids (8,63 and 5,75 %

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mass of dry residue respectively). The ratio of essential and non-essential amino acid 1:2 and this is near to the ratio in poultry meat.

In composition of lipid fractions of the additive there prevail polyunsaturd and monounsaturated acids Large share belongs to oleic acid ($C_{18}^{1=}$) – up to 35% from total; from polyunsauturated there prevail lenoleic ($C_{18}^{2=}$) – up to 21% (tab. 1).

The level of thyrosine accumulation at hydrolysis of proteins of food additive by digestive ferments is 57 mkg/cm^3 that quantitatively is near to the indict by pork digestibility.

The suggested modified technology envisages the addition of protein – fat additive with mass share 15% to the main raw material at the stage of cutting; the formation of a pattern on the cut from pieces of «white» and «red» bird meat; hand molding with thickness as back fat and not thicker than 5 mm and then in a casing.

The analysis of physicochemical property, organoleptic indices confirmed the fact that experiment samples of stuffed sausages with the substitution of the main raw material are similar to those prepared according to the traditional technology. The output of the experiment sausage «Donskaya» increases on 4% in comparison with the control one.

The results totality of investigations carried out all awed to

Table 1 - Fatty acid composition

Acid	Formula	Mass share of acids, % to total	
		Protein-fat emulsion	Poultry meat
Miristic	CH_3-$(CH_2)_{12}$-COOH	1,15	0,10
Palmitinic	CH_3-$(CH_2)_{14}$-COOH	25,96	26,00
Palmitoleic	CH_3-$(CH_2)_5$-CH=CH-$(CH_2)_7$-COOH	9,42	–
Stearic	CH_3-$(CH_2)_{16}$-COOH	6,06	6,00
Oleic	CH_3-$(CH_2)_7$-CH=CH-$(CH_2)_7$-COOH	34,89	24,00
Linoleic	CH_3-$(CH_2)_3$-$(CH_2$-CH=CH$)_2$-$(CH_2)_7$-COOH	21,12	21,00
Linolenic	CH_3-$(CH_2$-CH=CH$)_3$-$(CH_2)_7$-COOH	0,65	0,84
Other fatty acids in total		3,17	9,30

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recommend the food additive made of birds heads and legs produced by the technological method to use it in formulations of cans, stuffed sausages, half-finished products, culinary items, pastes in the quantity up to 15% instead of the main raw material. The treatment by ferment preparation allows to increase considerably the biological value of the additive, which in combination with the other raw material components can be recommended at the development of medicinal and prophylactic products, and products of special appointment.

Introduction of principles of the suggested scheme of carcasses cutting in production will allow to wider considerably the assortment of products prepared from poultry meat due to the production of non-traditional products, to use rationally the vain and secondary raw materials, to increase the output of ready – made products and the production potential of poultry processing industry.

It should be also mentioned the social meaning of the elaboration because the products are enriched with biologically active components correcting the human health.

As for the rational use of protein resources the production of keropeptides from feathers is rather interesting.

Good protein fat additive has high indices of emulsion stability, emulsifying, fat holding capacities in compassion with the initial raw material that contributes to a high thermal stability of the additive. As for the rest functional indicts the food additive approximates to the sausage meat at the substitution of the main raw material the food protein-fat additive in sausage meat has the role of emulsifier, waterbining and structure forming component.

The increase of the share of protein-fat additive in composion of a model sausage meat from 0 up to 15% leads to the increase of WHC, THC, WBC and stickiness. At 15% substitution of the main raw material the output of the ready-made product 2-4 % increase.

According to the investigations carried out the protein – fat additive from skin of land birds is recommended to the use technology of stuffed sausages. We elaborated modified formulations of stuffed sausages of the highest grade: "Usmanskaya", "Kurinaya", "Donskaya pharshirovannaya", "Appetitnaya" in accordance with the principles of the suggested scheme of rational cutting and poultry processing.

The production technology of keropeptides includes the following stages: preparation of feather - down raw material (defeating, washing and flusking); preliminary thermal treatment of the keratine protein in the presence of the chemical reagent for supturing of strong disulphide bonds in the keratine; fermentative hydrolysis with photolytic preparation up to complete solution of feather - down raw material; separation; vacuum - evaporation and spray drying.

The obtained experimental – industrial preparation is a powder with dispersity 0-h70 mkm, of lusterless color, with weekly expressed specific flavourl.

Chemical composition of the kerapeptide is disting guished by, a high mass share of the protein – 68,95-76,45 % and minerals – 10,37-14,96 %, besides mass share of

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moisture and fat is 6,60-7,23 and 0,08-0,09 respectively. Kerapeptide consists mainky of peptides and soluble proteins free amino acids compose 4,92 % (the content of essential amino acids - 30,70 %). The presence of peptides with molecular mass up to 5000 kDa, considerable content of cysteic acid in composition of amino acids causes the expediency of direct application of the kerapeptide in formulations of varios products.

Investigations demonstrate the presence of a high mass share of hyaluronic acid in composition of cocks combs. The treatment with ferments of the grinded raw material allows to isolate in purled form (admixture is about 1 %) and in the form of salts preparations which are widely known for their positive actions upon properties of skin in cosmetology.

Thus due to realization of rational use of the cutting scheme of broilers carcasses and ferment modification of the raw material it is possible to produce a wide range of food products biologically active ingredients including ones for functional diet.

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SYNERGISTIC ACTION OF MICROBIAL TRANSGLUTAMINASE AND HIGH PRESSURE ON LOW-FAT AND LOW-SALT CHICKEN GELS WITHOUT PHOSPHATES

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Keywords: chicken gels, high pressure, transglutaminase.

Abstract

Simultaneous application of transglutaminase (TGase) and high pressure (HP) has been found effective due to acceleration of the acyl transfer reaction. The objective of this study was to investigate the effect of microbial transglutaminase and HP applied to low-fat and low-salt chicken batters, containing egg components but no phosphates. Hardness and chewiness increased with increasing pressure. The higher springiness was observed in samples with TGase treated at 500 MPa. Differences in microstructure were clearly appreciated between samples with and without TGase. Pressurized samples were lighter and more reddish than cooked ones. In conclusion, a synergistic effect on texture, microstructure and colour of chicken gels was observed when HP processing and TGase were combined.

Résumé

L'application simultanée de transglutaminase (TGase) et de hautes pressions (HP) est efficace grâce à l'accélération de la réaction de transfert de groupements acyl. L'objectif de cette étude était d'étudier l'effet de la transglutaminase microbienne et des HP sur les propriétés de 'pâtes' depoulet additionnées d'ovoproduits, contenant de faibles teneurs en matières grasses et en selet sans phosphates. La dureté et la masticabilité augmentaient avec l'augmentation de la pression. La plus grande élasticité a été observée dans les échantillons contenant de la TGase et traités à 500 MPa. Des différences de microstructure ont été clairement observées entre les échantillons traités ou non à la TGase. Les échantillons pressurisés étaient plus clairs et plus rouges que les échantillons cuits. En conclusion, un effet synergique des

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traitement par HP et par TGase a été observé sur la texture, la microstructure et la couleur des gels préparés à base de viande de poulet.

Introduction

Gels made under high pressure (HP) are generally softer than those obtained by conventional heat treatment (Jiménez Colmenero, 2002).Transglutaminase (TGase) is widely accepted for improving functional properties of meat products. Simultaneous application of TGase and HP was found to be effective at accelerating the acyl transfer reaction (Nonaka *et al*., 1997). This reaction causes crosslinking of protein molecules and results in important physical changes. Ashie and Lanier (1999) enhanced the effects of HP on surimi and turkey breast muscle gelation with the addition of TGase. The texture of chicken sausages was improved by addition of soybean and milk proteins modified by TGase at reduced levels of phosphates (Muguruma *et al*., 2003). In a previous study, we have shown that the addition of yolk and egg white together with TGase and HP improved texture and water holding capacity of chicken gels. The objective of this study was to investigate the synergistic effect of microbial TGase and HP applied to low-fat and low-salt chicken batters, containing egg components but no phosphates.

Material and Methods

Preparation of low-fat and low-salt chicken gels

Fresh chicken thighs and eggs were purchased from a local market. Skinless, boneless meat was trimmed to remove visible fat and connective tissue, ground twice through 6 and 3 mm plates in a mincer Mod. PC-22, (Sammic, SA, Spain), then mixed with NaCl (1.0% w/w) and left standing 18 hr at 4ºC. The ground meat was homogenised with dehydrated ovalbumin 10% (Degussa Texturants), 10% of fresh egg yolk and cold water (30%) in a Stephan UMC 5, (Stephan u. Sohne, GmbH & Co, Germany) at 1800 rpm and 80% vacuum for 12 min. Final temperature of the batters never exceeded 10 ºC. Samples with enzyme were added with 0.3% TGase Activa WM (Ajinomoto Co. Inc, Japan). Immediately after homogenate preparation, batters were stuffed (Mod. TWF-6, Dick GmbH, Germany) into 22 mm ∅ cellulose casings (Nojax, Viskase SA) and polyvinilidene casings of 55 mm ∅ (Krehalon, Holland). Samples were vacuum packaged in a Packaging VS 26 (Cryovac, Switzerland) before treatments.

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Pressure and thermal treatments

Batters with TGase were treated 15 min after manufacturing by pressure or by heat. Samples were pressurized at 500, 700 and 900 MPa at 40 ºC during 30 min in a discontinuous high pressure pilot unit "Food-Lab" S-FL-850-9W (Stansted fluid Power, Ltd, UK). All pressurized samples were heated in a water bath to allow an internal temperature of 75 ºC for 5 min to inactivate the enzyme and, thereafter, cooled down in cold water until 20 ºC. A standard cooking process at 75 ºC for 30 min was applied to non pressurized batters (0.1MPa). Samples without enzyme were treated in the same conditions. The gels were stored at 4ºC until further analysis.

Yield determination

The yield of gels was calculated as the weight of samples heated or pressure treated divided by the weight of the non treated samples, expressed as a percentage.

Proximate analysis and pH

Moisture, ash and total nitrogen of raw meat, batters and gels were determined by duplicate (AOAC; 1984). Fat content was evaluated by difference. The pH was determined by triplicate in a pHmeter MicropH 2001 (Krison, Spain) on a homogenate of 5 g sample in 50 ml distilled water at 20 ºC.

Coluor determination

Colour of gels was measured with a portable spectrocolorimeter MiniScan XE™ (Hunter Ass. Lab., Inc., VA.) and expressed as L (lightness), a (redness) and b (yellowness). Six cylindrical replicates (5 cm ∅) were cut to analyse the internal colour of gels. Measurements were done with reference to iluminant Fcw and the 10º standard observer.

Texture

Textural characteristics of gels were analysed according to the texture profile analysis (TPA) (Bourne, 1978) and cutting force using a TA-XT2 Texture analyser (Stable MICRO Systems, UK) with a 25 kg load cell. Four replicates were cut (22 mm diameter and 20 mm height) and axially compressed twice to 40% of their original height at a crosshead speed of 1mm/seg with an aluminium compression platen (5 cm diameter).

Confocal scanning laser microscopy

A confocal scanning laser microscope Leica DM IRE 2, (Leica GmbH, Germany) was used to observe the microstructure of gels. A 0.1% aqueous acridine orange solution mixed with an equal volume of 1% acetic acid was used to stain the gel. Samples were excited at 514 nm with a Ar/He Ne laser.

Statistical analysis

Results were analysed using ANOVA with the General Linears Model Procedure of The SAS© System for Windows V8 (SAS Institute Inc, USA). Level of significance was set for $P < 0.05$. Differences among means from each variable and treatment were determinated using Studentized Maximum Modulus (GT2) Test.

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Results and discussion

Proximate composition and pH of chicken meat, batters and gels are shown in Table 1. Samples pressurized without TGase had a higher yield (99.3 to 99.7%) than those with TGase (94.7 to 95.8%) and samples treated by heat (96.04 to 97.9%). These percentages are similar to those reported by Pietrasik (2003) in beef gels with egg albumin and phosphates in cooked samples.

Colour results are shown in Table 2. Pressurized samples showed higher L values at 900 and 700 MPa, but samples treated at 500 MPa were darker than cooked ones. The samples treated at 500 MPa with TGase showed the highest a and b values, and pressurized samples were redder than cooked ones due to the partial oxidation of ferrous myoglobin into ferric metmyoglobin. There were no differences in samples treated at 700 and 900 MPa with or without enzyme in redness but addition of TGase increased yellowness. Between cooked samples, there were no significant differences in lightness and redness, but samples with TGase had lower yellowness. Pietrasik (2003) reported that TGase addition had no significant influence on the L and a colour parameters of experimental beef heat-gels although he observed that the presence of egg albumen caused an increase in yellowness when enzyme is added. Tseng *et al*. (2000) found that 1% pig plasma TGase did not produce significant differences in colour of low salt chicken meat balls cooked at an internal temperature of 71ºC and Kilic (2003) reported non significant differences between samples of döner kebab added with TGase and sodium caseinate.

Pressurized samples with TGase addition were significantly harder and presented higher chewiness than the other ones, likely as a consequence of crosslinks formed between chicken meat and egg proteins (Table 3). There were no differences between cooked samples with or without TGase, certainly because of the inactivation of the enzyme at cooking temperature (75 ºC for 30 min). The effect of TGase on texture was observed by Pietrasik (2003) in samples treated by heat. Beef gels exhibited greater hardness, cohesiveness, springiness, and chewiness than those produced without TGase. Furthermore, the cohesiveness was increased only in samples containing egg albumen. Samples treated by high pressure and with enzyme had more springiness than cooked samples and those produced at 500 MPa shown highest values. Samples with TGase treated at 700 and 900 MPa were more cohesive than those treated by heat. Samples obtained by high pressure without TGase showed similar pattern on texture parameters than those with TGase, but in a lower scale.

Microstructure of samples were correlated with results of textural parameters. The samples obtained with addition of TGase had a firmer gel and homogeneous network structure obtained by the formation of intramolecular and intermolecular crosslinks between myofibrillar and egg proteins.

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Conclusions

A clear synergistic effect on texture, microstructure and colour of chicken gels was observed when high pressure processing and transglutaminase were combined, although the effect of 900 MPa was no significantly different from 700 MPa in samples with or without enzyme.

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Acknowledgments

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Table 1. Proximate Analysis and pH of meat, batters and gels

Sample	Moisture %	Ash %	Protein %	Fat %	pH
Raw chicjken meat	74.10	1.11	20.76	4.03	6.98
Chicken batters	70.81	2.12	21.06	6.01	7.67
Chicken gels NE	70.02	2.17	19.22	8.59	6.82
Chicken gels TG	69.60	2.27	19.50	8.63	6.81

NE=Without Transglutaminase. TG=Transglutaminase added

Table 2. Hunter color values of low-fat chiken gels

Sample	MPa	ºC	L		a		b	
NE75	0.1	75	77.09 ±0.33	b	2.64 ±0.04	d	14.96 ±0.16	b
NE500	500	40	72.35 ±0.45	c	4.20 ±0.27	b	15.24 ±0.15	b
NE700	700	40	76.91 ±0.62	b	3.22 ±0.24	c	13.58 ±0.13	d
NE900	900	40	78.08 ±0.35	a	3.16 ±0.06	c	13.49 ±0.08	d
TG75	0.1	75	76.45 ±0.62	b	2.35 ±0.14	d	14.51 ±0.56	c
TG500	500	40	74.77 ±0.28	c	5.14 ±0.31	a	16.56 ±0.11	a
TG700	700	40	78.22 ±0.15	a	3.25 ±0.02	c	15.39 ±0.15	b
TG900	900	40	78.07 ±0.17	a	3.21 ±0.04	c	15.31 ±0.08	b

NE=Without Transglutaminase. TG=Transglutaminase added.
Means with different letters in the same column are significantly different.($P<0.05$)

Table 3. TPA parameters of low-fat chicken gels.

Sample	MPa	ºC	Hardness (N)		Springiness (mm)		Cohesivity		Chewiness (N x mm)	
NE75	0.1	75	36.05 ±0.59	c	6.995 ±0.24	b	0.538 ±0.00	cd	135.77 ±4.09	c
NE500	500	40	33.18 ±1.34	c	7.397 ±0.20	ab	0.515 ±0.00	f	126.48 ±5.88	c
NE700	700	40	36.70 ±2.41	c	7.322 ±0.19	ab	0.545 ±0.00	c	146.63 ±9.65	c
NE900	900	40	36.19 ±1.39	c	7.255 ±0.13	ab	0.547 ±0.00	c	143.61 ±3.25	c
TG75	0.1	75	33.92 ±1.93	c	7.030 ±0.33	b	0.533 ±0.00	de	126.96 ±5.63	c
TG500	500	40	44.63 ±1.45	b	7.575 ±0.21	a	0.527 ±0.01	e	178.02 ±4.68	b
TG700	700	40	55.43 ±4.05	a	7.322 ±0.08	ab	0.573 ±0.00	a	232.85 ±16.39	a
TG900	900	40	57.58 ±2.62	a	7.327 ±0.14	ab	0.558 ±0.00	b	235.50 ±9.29	a

NE=Without Transglutaminase. TG=Transglutaminase added.
Means with different letters in the same column are significantly different. ($P<0.05$)

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BIOLOGICAL VALUE INCREASE OF CHICKEN-BROILERS MEAT

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Keywords: Enzymes hydrolysis, collagenase, chicken-broiler, meat, water- and salt soluble protein fraction.

Abstract

It was investigated water- and salt soluble protein fraction of chicken-broilers white and red meat by collagenase enzymes preparation (CEP). It was revealed that protein hydrolysis degree didn't exceed 40 % due to the impact restriction of the CEP in accordance with its peculiarities on some defined peptides bonds break. Full CEP inactivation wasn't observed. Proteolitic activity reduction of the enzymes preparation was only 19.1-38.5 % in the both fractions. Amino-acid concentration of different protein fraction increased more than in 2 times due to CEP application.

Résumé

A l'issue d'une préparation avec des enzymes de type collagénase (CEP), nous avons étudié les fractions protéiques solubles dans l'eau et le sel, de la viande blanche et rouge de poulet. Le degré d'hydrolyse des protéines n'a pas dépassé 40 % du fait des caractéristiques de CEP. Une inactivation complète de CEP n'a pas été observée. La diminution de l'activité protéolytique de la préparation enzymatique était de 19.1-38.5 % dans les deux fractions. La concentration en acides aminés des différentes fractions protéiques a été multipliée par plus de 2 après le traitement CEP.

Introduction

One of the most perspective directions of biotechnology is enzymes application in food-processing industry, including meat branch. According to some specialists opinion the basic and the secondary raw material enzymatic treatment is an important

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factor of the technological processes innovations and new food sources and products manufacture. It should be noted that this very approach is of great theoretical and practical value [1, 2].

Our country and foreign experience of enzymes practical application has demonstrated that in the meat industry enzymes preparations are effectively used for the purpose of tough meat softening, cooked and salt sausage products quality improvement, different protein enrichers and food additives production and blood clarification.

The proteolytic enzymes preparations give an opportunity to produce good quality meat products with a rather high degree of assimilation. Partially hydrolyzing protein, enzymes promote actin-myosin complex protein solubility, albuminous molecule hydration increase, connected water contents and the product output increase.

Moreover enzymes preparations application as additives during meat corning improves finished product consistency and digestibility [4].

The purpose of the investigation was to evaluate qualitative and quantitative characteristics of water- and salt soluble protein fraction of the second category chicken-broilers white and red meat under the influence of collagenase enzymes preparation from hepatopancrease of Kamchatka crab.

Material and Methods

The object of the investigation was the second category chicken-broilers white and red meat from the Joint-Stock Company “Chicken Kingdom” of Lipetsk city. The substrates for the hydrolysis dynamic determination were water- and salt soluble meat protein fractions produced by distilled water and Veber salt solution consecutive extraction. As an enzyme it was used a collagenase industrial preparation in concentration of 40 units proteolytic activity on 1 gr of protein in the extract which provides a maximal degree achievement of the fraction hydrolysis. Enzymes hydrolysis was carried out on installation of UVMT-12-250 at 40 ^{o}C, pH= 7,0-7,2 and continuous agitation within 6 hours. During this process following indexes were determined: protein mass portion with the help of biuret –reaction, peptides and amino-asids mass portions according to the ninhydrine-method, proteolytic activity of the enzymes preparation and tyrosine amino-asid mass portion at the lateral bonds break formed by its phenolic ring [6].

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Results and discussion

The results of the investigation have shown that the water- and salt soluble protein fraction hydrolysis of white and red chicken-broilers meat by CEP was the most intensive during the first three hours. The protein fractions were in soluble condition and in initial moment they constitute 4.75-5.75 mg/cm^3 of protein. During the hydrolysis the soluble protein was decomposed by enzyme up to low-molecular products, soluble protein mass portion has been decreased to the values of 3,26-4,50 mg/cm^3 by 3 hours of treatment. The further substrate incubation by collagenase enzymes preparation wasn't of great importance. The soluble protein mass portion reduction was less than 0.2 mg/cm^3. Soluble protein in the fraction has been hydrolysed on 24.1-39.4 %. It was revealed that the hydrolysis values for white meat were 33.3-39.4 % and for red meat – 24.1-26.3 %.

The process of the hydrolysis was accompanied by continuous peptides and amino-acids increase (fig. 1b, 2b). Low-molecular products mass portion was 1450-3250 mkg/cm^3. Peptides and amino-acids accumulation was carried out during the whole experiment. Their mass portion has been increased in 1.5-2.0 times. The most intensive increase of these products was evident for the salt soluble fraction of red meat. It was from 1450 to 2920 mkg/cm^3.

It was found that the enzymes hydrolyse distinctive feature of water- and salt soluble fraction of white and red meat was soluble protein decomposition to peptides and amino-acids without tyrosine amino-acid free radicals formation. In this case, CEP influences on peptide bonds in protein fraction without release of the lateral bonds, formed by tyrosine amino-acids.

Results comparison of the fraction protein hydrolysis and amino-acids and tyrosine concentration increase has proved irregular feature of substrate decomposition, i.e. intensive hydrolysis is observed in the first hours, then it becomes less after 3-4 hours and stops by the end of the experiment. This fact may be due to the full CEP inactivation or it may be caused by impact restriction of the CEP in accordance with its peculiarities on the substrate hydrolysis.

This assumption has been checked up by the preparation proteolytic activity during the hydrolysis of water- and salt soluble protein fraction of white and red chicken-broilers meat. The CEP activity of the analyzed fraction after 6 hours was as follows:

- water- and salt soluble fractions of white meat were 80,9 and 66,0 correspondingly;
- water- and salt soluble fractions of red meat were 66,9 and 61,5 correspondingly.

According to these results the full enzymes preparation inactivation wasn't observed. Proteolytic activity reduction was marked only on 19,1-38,5 % in protein fractions. Hence, protein hydrolysis in the fractions did not exceed 40 % due to the impact restriction of CEP in accordance with its peculiarities on some defined peptides bonds break.

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So, CEP application is very important for the production of protein hydrolisates with a high mass portion of soluble protein. Peptides and free amino-acid increase in the examined samples may lead to the product digestibility improvement. Such products are recommended for people suffering from gastric and intestinal diseases and for sportsmen after their intensive trainings.

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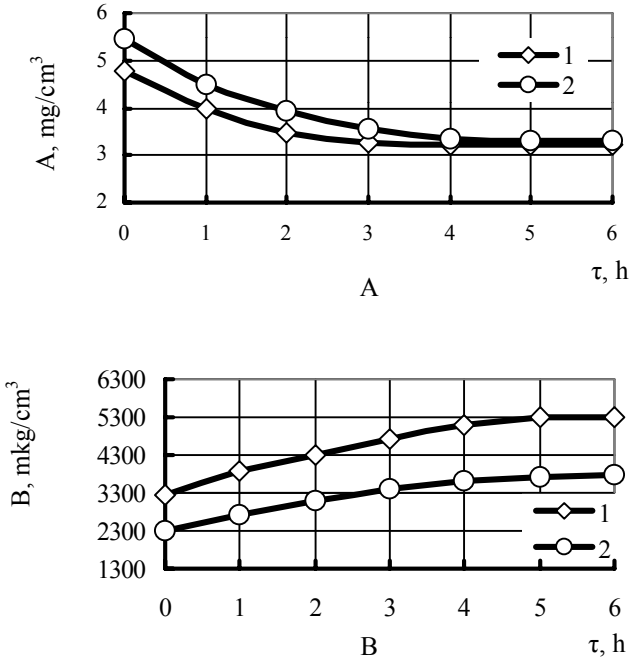

Fig. 1 Enzymatic hydrolysis of chicken-broilers white meat protein fraction by CEP:

1 – water soluble fraction; 2 – salt soluble fraction;
A – protein concentration, mg/cm^3;
B – lysin concentration, mkg/cm^3;
τ – process duration, hours

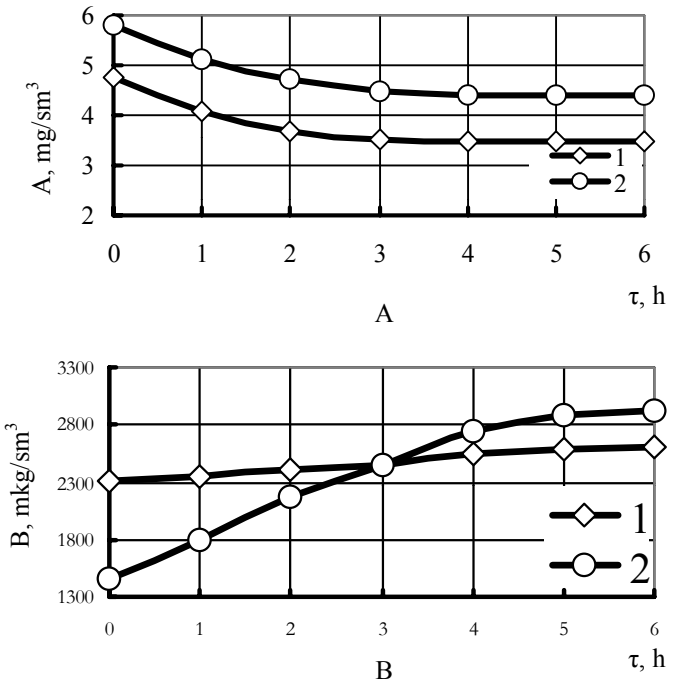

Fig. 2 Enzymatic hydrolysis of chicken-broilers red meat protein fraction by CEP:

1 – water soluble fraction; 2 – salt soluble fraction;
A – protein concentration, mg/cm^3;
B – lysin concentration, mkg/cm^3;
τ – process duration, hours

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EARLY POSTMORTEM INJECTION AND TUMBLE MARINATION EFFECTS ON QUALITY AND SODIUM PENETRATION OF BROILER BREAST FILLETS

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Keywords :Tenderness, Broiler, Marination, Phosphate, Meat

Abstract

Processors utilize in-line injection and tumble marination to decrease labor and handling. Stimulating pre-rigor muscle may cause increased toughness. This study compared marination of early postmortem (PM) breast meat using injection, vacuum tumbling, or both. From 45 broiler carcasses at 3 h PM, breast fillets were deboned and injected (10%w/w, 0.54%NaCl + 0.42%PO_4), tumbled (30min, 635mmHg, 14RPM), or injected + tumbled. Non-marinated controls deboned at 3h and 6h postmortem were included. Tumbled and control 3h fillets had higher shear value and cooking losses than the injected or tumbled+injected treatments. Sarcomere lengths were not different among 3-hr treatments. Tumble marination at 3hPM without injection produced tougher meat than injection, possibly due to stimulation, less brine distribution, or decreased fiber disruption as in the injected fillets. To test the brine penetration throughout the fillet, breast fillets from 60 broilers were deboned at 3hrPM and vacuum tumbled (30min, 635mmHg, 14RPM) with 15%w/w solution of 0.54%NaCl and 0.42%PO_4. Although there was no differences in shear value due to marination, cooked meat moisture and sodium were higher in the brined fillets. Sodium ion concentration was higher on the surface and on the epimysium side, suggesting some uneven distribution.

Résumé

Les processeurs utilisent l'injection et la marinade par 'barattage' pour diminuer les manipulations. La stimulation du muscle en pré-rigor peut augmenter la dureté de la

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viande. Cette étude compare les méthodes de marinade des filet tôt postmortem : soit par injection, soit par 'barattage' sous vide, soit en combinant les deux. A 3 h PM, les filets de 45 carcasses ont été désossés puis injectés (10%w/w, 0.54%NaCl + 0.42%PO_4), 'barattés' (30min, 635mmHg, 14RPM), ou injectés + 'barattés'. Des témoins désossés à 3 ou 6 h PM non marinés ont été inclus dans l'analyse. Les filets 'barattés' ou contrôles désossés 3 h PM étaient plus durs et perdaient plus d'eau à la cuisson que les filets injectés ou 'barattés injectés'. La longueur des sarcomères était la même pour tous les filets désossés 3 h PM. A 3 h PM, la marinade par 'barattage' donne des viandes plus dures, sûrement en raison de la plus forte stimulation du muscle, la moins bonne distribution de la saumure et une proportion moindre de rupture des fibres. La pénétration de la saumure a été testé sur 60 filets désossés à 3 h PM et 'barattés sous vide' (30min, 635mmHg, 14RPM) avec 15%w/w d'une solution de 0.54%NaCl et 0.42%PO_4. Bien que la marinade n'affecte pas la tendreté de la viande, les teneurs en eau et en sodium étaient plus élevées dans les filets saumurés. La concentration en sodium était supérieure en surface, spécialement du côté de l'epimysium, suggérant une distribution inégale de la saumure dans le muscle.

Introduction

Marination of broiler breast meat has become an integral part of the poultry industry and production efficiencies have made in-line injection of fillets common, sometimes with detrimental tenderness effects. Dispersion of ions into the muscle provides a tenderizing effect due to the repulsion caused by association of the ions with the proteins. This repulsion also increases water uptake and therefore increases moisture content of the cooked meat and tenderness. However, incomplete dispersion of the ions through the meat can possibly affect the water holding capacity and tenderization potential of the marinades. Xiong and Kupski (1999) used a dye tracing method to determine penetration of the marinade into tumbled broiler breast fillets; however, the water soluble dye may migrate at a different rate from the ions which are responsible for increased tenderization. Also, these methods did not use a vacuum when tumbling the fillets, possibly affecting marinade penetration into the meat. Since there have been few studies relating early postmortem marination and tenderness, the objectives of these studies were to determine tenderness differences with several in-line marination techniques and to determine sodium ion migration through vacuum tumbled fillets to determine if complete dispersion occurs.

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Materials and methods

Experiment 1

Broiler breast fillets were deboned at 3 or 6 h postmortem (PM) from 45 carcasses in each of two trials. Treatments included injected (0.172 MPa, 10%), vacuum + tumbled (635 mm Hg, 14 RPM, 10%), injected + tumbled, and non-marinated controls for fillets deboned at 3 h PM. A post-rigor, non-marinated control deboned at 6 h PM was also included. The fillets were marinated with a 10% solution at a final concentration of 0.54% NaCl and 0.42% PO_4. All fillets were then stored at 4 C for 16h to allow for marinade equilibration. The fillets were then cooked and analyzed for Allo-Kramer shear, cook loss (%), and sarcomere length using the laser diffraction method. Data was analyzed by ANOVA and Duncan ($P \leq 0.05$).

Experiment 2

At 3h postmortem, fillets from 30 broiler in each of two trials were either untreated controls or were marinated with vacuum tumbling (635 mm Hg, 14 RPM) using a 15% solution of 0.54% NaCl and 0.42% PO_4. All fillets were allowed to equilibrate for 16h at 4C. The fillets were then cooked and measured for Allo Kramer shear and cooked meat moisture (%) by the oven-dry method. Two cores (2.54 cm diameter) were cut from the center of the fillets, sliced in 2mm thick slices, homogenized in deionized water and analyzed for sodium ion concentration using an ion selective sodium probe. Percent sodium ion was calculated for each slice based on the weight of each slice. Data were analyzed by ANOVA and Duncan ($P \leq 0.05$).

Results and discussion

Experiment 1

The vacuum + tumbled and control 3 h fillets had the highest shear value followed by injected + tumbled and injected which were not significantly different (Table 1). The injected fillets were significantly more tender than the tumbled fillets, possibly due to greater fiber disruption from fluid pressure, more extensive brine penetration and distribution, and the actual injector needles decreasing the integrity of the fiber network. Even though both tumbling and pre-rigor injection act as stimulation to the muscle, the fiber disruption maybe greater in the injected fillets, decreasing toughness associated with pre-rigor muscle stimulation. Tumbling fillets in the pre-rigor state may increase the physical stimulation of the muscle and cause increased toughening. Also, the penetration and distribution of the marinade may be less in the tumble-only fillets when compared to the fillets that had the brine injected into them.

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The longer sarcomeres observed in the control 6h fillets were expected since rigor mortis had developed before the muscle was removed from its skeletal restraint. The lack of differences between the injected and tumbled fillets is inconsistent with the shear value results but suggests that sarcomere length is just one factor influencing tenderness. Other factors such as hydration and protein interaction may also be involved. There were no significant differences in cooking loss between any of the 3 h treatments. The numerical distinctions between these means may simply reflect the variable nature of measuring small weight changes in individual fillets. Although the magnitude of these mean differences may be large enough to be practically important, statistical conclusions from this data set may not be possible.

***Experiment* 2**

There were no significant differences in shear value between control and marinated fillets (Table 2). Even though sodium ions have a tenderizing effect in post rigor broiler breast meat, the early postmortem stimulation caused by early deboning and tumbling in the present experiment may have masked this effect. Tumbled fillets had higher cooked meat moisture than control fillets (Table 2).

Sodium ion concentration was greater in the treated than the control fillets and was greater on the surfaces of the treated fillets than in their center (Figure 1). These differences indicate that sodium ions migrate from both the lateral and medial surfaces to the center of the fillet. The significantly higher sodium ion concentration on the lateral side of the treated fillets compared to the medial side of the fillet may be due to the presence of the epimysium on the lateral surface of the fillet.

The results of these studies indicate that in-line tumble marination at 3 h PM without injection produces significantly tougher meat than the injection treatments, possibly due to decreased disruption of fibers and decreased penetration and distribution of the marinade as occurs in the injected fillets. Also, vacuum tumbling pre-rigor broiler breast meat allows for sodium ion penetration to the center of the fillet but with increased concentrations on the surfaces of the fillet.

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TABLE 1. Raw sarcomere length, cook loss, and cooked meat shear value of broiler breast fillets (Experiment 1)

Parameter Measured	Treatments					
	Control 3 h PM[1]	Inject 3 h PM	Tumble 3 h PM	Inject+Tumble 3 h PM	Control 6 h PM	Pooled SEM
Shear Value (kg/g)	14.09[a]	7.81[b]	14.49[a]	9.05[b]	5.34[c]	0.04
Sarcomere Length (μm)	1.60[b]	1.61[b]	1.56[b]	1.63[b]	1.80[a]	0.01
Cook Loss (%)	23.58[ab]	22.58[ab]	26.30[a]	26.78[a]	19.56[b]	0.03

[a,b]Means in a column differ ($P < 0.05$)
[1]Postmortem deboning time.

TABLE 2. Shear value and moisture content of cooked broiler breast fillets (Experiment 2)

Parameter Measured	Treatments		
	Control 3 h PM[1]	Tumble 3 h PM	Pooled SEM
Shear Value (kg/g)	13.23	12.21	0.64
Moisture (%)	70.31[a]	71.46[b]	0.21

[a,b]Means in a column differ ($P < 0.05$)
[1]Postmortem deboning time.

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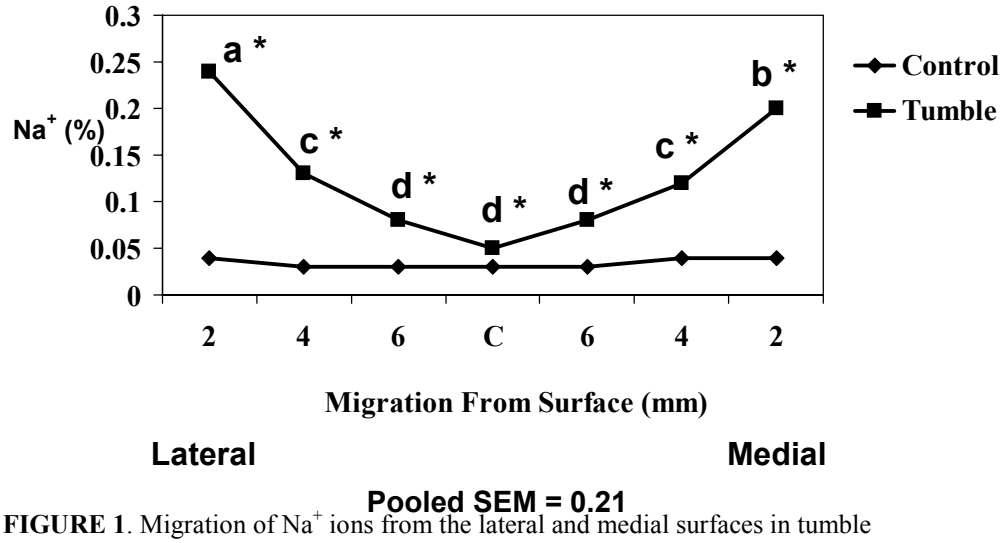

FIGURE 1. Migration of Na^+ ions from the lateral and medial surfaces in tumble marinated broiler breast fillets (14mm). a, b, c, d = $P < 0.05$ indicated differences between slices. * = $P < 0.05$ indicates difference within a treatment.

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CHARACTERISTICS OF FERMENTED TURKEY ROLLS PREPARED USING CULTURES OF *PEDIOCOCCUS ACIDILACTICI* AND *MICROCOCCUS VARIANS*

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Abstract

Fermented turkey rolls were prepared using *Pediococcus acidilactici* injection and *Micrococcus varians* addition during mixing. Semi-dry and non-dried products after 14-16 days at 2 C differed in composition and yields but not shear values. Fermented and non-fermented control rolls also differed in pH, acidity and percent cured pigment content. Sensory preferences showed that control product scored better by panelists than product fermented with *P. acidilactici* alone. Lactic acid bacteria survived in products at 4.3-4.6 $\log_{10}$ CFU/g for non-fermented rolls and 3.7-4.3 $\log_{10}$ CFU/g for fermented products. Reductions of initial psychrotrophic and staphylococci populations were higher for fermented and dried product as compared to non-fermented controls likely due to produced acidity.

Résumé

Des rouleaux de dinde fermentés sont préparés par injection de saumure contenant *Pediococcus acidilactici* avec ou sans *Micrococcus varians.* Des témoins sont préparés sans addition de ferments. Les témoins et les produits fermentés sont divisés en deux lots : un séché et un non-séché et sont conservés à 4°C pendant 14-16 jours. En fin de conservation, les produits séchés et non-séchés diffèrent en composition, en rendement mais sont identiques pour les forces de cisaillement. Les témoins et les produits fermentés diffèrent en acidité, pH et pourcentage de pigments saumurés. Sur le plan senroriel, les témoins sont jugés meilleurs que les produits fermentés avec *P. acidilactici.* Les bactéries lactiques survivent dans le témoin à raison de 4.3-4.6 $\log_{10}$ CFU/g et dans les produits fermentés à raison de 3.7-4.3 $\log_{10}$ CFU/g. Une réduction supérieure de populations psychrotrophe et de staphylocoque est notée dans les produits fermentés, certainement en relation avec l'acidité de ces produits.

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Introduction

The majority of fermented meat products in the market today are semi-dry or dry sausages and relatively few types are made with poultry meat. As sausages, the significance is that the meat is coarsely to finely ground and this makes it easy to incorporate ingredients and get good distribution. Fermented products made with whole pieces or sections of muscle tissues are very limited and this offers new product opportunities. However, distribution of the starter culture and fermentable carbohydrate substrate is not easy and requires injection coupled with adequate mixing. The objective of this study was to compare compositional, sensory and microbiological properties of non-fermented and fermented turkey rolls prepared from whole and sectioned, non-ground turkey thigh pieces. Both non-dried and semi-dry products were evaluated for most characteristics.

Materials and Methods

Boneless turkey thigh meat was injected with curing brine containing *P. acidilactici* with and without addition of *M. varians* during mixing. A control with no culture was also prepared. After blending under vacuum for 15 min, inoculated mixes were stuffed in 7.6 cm diameter fibrous casings and fermented at 34 C and 95-98% RH for 15-17 h while the non-fermented control mix was held at 2-4 C for the same period. The turkey rolls were heated starting at 65.5 C with final holding at 90.5 C until an internal temperature of 74 C was attained (5.0-5.5 h total). The stuffed rolls were then cooled to 25 C using a cold water spray. One-half of the product was dried at 8 C at 80-90% RH to an approximate weight loss of 15% (3-4 days required) and then vacuum packaged and held at 4 C for 11-12 days. The other half of the rolls was vacuum packaged and held at 4 C for 14-16 days.

From initial weights of stuffed casings, product yields were determined using weights at 14 days post-processing. Moisture, fat, protein (N x 6.25) and ash contents were determined by AOAC (1995) procedures and NaCl was determined using Quantab Chloride Titrators (AOAC, 1990). Product pH and lactic acid content were determined following the procedure of Korel and Acton (2002) and percent of total pigment existing as the cured nitrosylhemochrome pigment was determined by the procedure of Acton and Dick (1977). Shear force as kg/g-cm^2 surface area for 3 mm thick slices of 7 cm diameter product was measured using a Kramer multi-blade shear cell attached to an Instron 122 Universal Testing Machine. An 18-member sensory panel scored preference rank on a 4-point scale (1=most preferred to 4=least preferred) in 4 sessions

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following the 14th day of product storage. Panelists were familiar with the type of product based on preliminary discussion and evaluation training sessions.
Lactic acid bacteria, psychrotrophic counts and staphylococci were determined using the following isolation media and incubation conditions : MRS broth with agar (32 C in 0.4L/h CO_2 for 48-72 h), plate count agar (10 C for 7 days) and Baird Parker agar with egg tellurite (32 C for 24-48 h), respectively. A 25 g sample from the interior of the turkey roll was blended in a stomacher with 225 ml of physiological saline, serial dilutions made and then pour plated and incubated. Bacterial counts were expressed as $\log_{10}$ colony forming units (CFU) per g sample.

Results and discussion

Overall compositional profiles (Table 1) of the non-dried and semi-dry turkey rolls are similar to results reported for turkey ham (non-dried) and fermented, semi-dry turkey sausage (Acton et al., 1979; Acton and Keller, 1976). Yields reflected weight losses during fermentation and heating to 74 C for the non-dried product and the additional loss that was expected during dehydration for the semi-dry turkey rolls. These yields are identical to those reported by Terlizzi et al. (1980) in similar processing of boneless ham from pork meat. No difference was found in shear values, likely due to the finer fiber structure of turkey muscle in comparison to the increase of shear values normally found in drying of fermented pork and beef products which have larger fibers.
Acidities of the fermented turkey rolls (Table 2) were in the lower range of 0.5-1.5% lactic acid typical of fermented sausages (Korel and Acton, 2002) yet were similar to acidities for fermented and dried sectioned-and-formed hams from pork (Terlizzi et al., 1980). The pH range of 5.18-5.26 is within the upper part of the pH range of 4.8-5.4 generally found in similar products of red meats (Korel and Acton, 2002). The development of less acidity and a pH higher than pH 5.0 for the fermented turkey rolls may be due to differences of fermenting larger intact muscle pieces (thigh) as compared to smaller ground tissue particles typical of fermented sausages.
From panel sensory data (Table 2), preference scores of the non-dehydrated turkey roll products ranked in the following order: non-fermented control product ≥ fermented, semi-dry *P. acidilactici* plus *M. varians* product ≥ fermented *P. acidilactic* single culture product. Non-fermented product received 2.2 to 3.4 times as many panelist ratings for “most preferred” sample than was received by samples of the fermented products (dual culture and single culture, respectively). Use of starter cultures for fermentation decreased overall preference. These results do not agree with previous studies of fermented sausages that show higher preference scores as lactic acid concentrations increase (Deibel et al., 1961; Acton and Keller, 1974).

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Initial bacteria counts of the raw, boneless turkey thigh meat for lactic acid bacteria, psychrotrophs and staphylococci were 3.47, 2.99, 2.90 $\log_{10}$ CFU/g, respectively. Inoculation with starter cultures for the mixes to be fermented increased lactic acid bacteria counts to 7.5-7.8 $\log_{10}$ CFU/g. Final products, after heating and drying (for semi-dry rolls), had lactic counts ranging from 3.7 $\log_{10}$ CFU/g (fermented, semi-dry) to 4.6 $\log_{10}$ CFU/g (non-dried control). The counts for remaining lactic acid bacteria are in agreement with previous studies where heating was less than that for fully cooked products attaining 76-82 C internally. After heating, only 8 of 26 samples (30.7%) were positive at > 1.00 $\log_{10}$ CFU/g of psychrotrophs and 6 of those were for non-fermented, semi-dry rolls. For staphylococci, 4 of 26 samples (15.4%) had > 1.00 $\log_{10}$ CFU/g and 3 of those were non-dried, non-fermented rolls. Survival of staphylococci at levels found in these products would probably not pose a public health problem. However, staphylococcal enterotoxin production was not tested in these products.

This study does indicate that no sensory benefit was achieved by fermenting the turkey rolls. However, an increased acidity associated with fermentation, when coupled with heating during processing, may slightly contribute to improving the microbiological quality of this type of fermented poultry product.

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Table 1. Composition, product yield and shear value of non-dried and semi-dry turkey rolls.

Constituent (%)	Nondried	Semi-dry
Moisture	71.5 a	66.6 b
Fat	5.5 a	5.6 a
Protein	22.6 b	24.4 a
Ash	3.0 b	3.4 a
NaCl	2.09 b	2.14 a
Yield	87.1 a	83.2 b
Shear*	0.056 a	0.063 a

[a-b] Row means not having a common letter differ (P<0.05).
* Shear value in kg/g-cm^2

Table 2. pH, lactic acid, cured pigment and sensory preference scores for nonfermented and fermented turkey roll products.

Measure	Nonfermented (Control)	Fermented P. acidilactici	Fermented P. acidilactici plus M. varians
pH			
Nondried	6.41 a	5.26 b	5.18 c
Semi-dry	6.39 a	5.25 b	5.21 c
Lactic Acid (%)			
Non-dried	0.04 b	0.59 a	0.62 a
Semi-dry	0.04 b	0.59 a	0.63 a
Cured Pigment (%)			
Non-dried	69.0 b	81.2 a	81.8 a
Semi-dry	54.0 b	76.3 a	81.3 a
Sensory			
Preference	2.82 a	2.15 b	2.48 ab
Most Preferred (n)	41	12	19

[a-c] Row means not having a common letter differ (P<0.05).

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Table 3. Bacterial counts (log_{10} CFU/g) of nonfermented and fermented turkey roll products.

Bacterial Group	Nonfermented (Control)	Fermented with *P. acidilactici*	Fermented with *P. acidilactici* plus *M. varians*
Lactic Acid			
Nondried	4.56 x	4.21 x	4.28 x
Semi-dry	4.34 x	3.68 y	3.73 y
Psychtrophs			
Non-dried	<1.00	<1.00	<1.00
Semi-dry	1.05	<1.00	<1.00
Staphylococci			
Non-dried	1.90	<1.00	<1.00
Semi-dry	<1.00	<1.00	<1.00

[a-c] Row means not having a common letter differ (P<0.05).

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PROPERTIES OF CHICKEN SALAD SPREADS PREPARED WITH VARIOUS LEVELS OF TEXTURED SOY PROTEIN

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Keywords : chicken salad, soy protein, quality, sensory aroma

Abstract

Chicken salad spreads were prepared with cooked chicken and unflavored or flavored textured soy protein (TSP) at 0-100% (25% increments). Of chicken-TSP combinations, total GC-MS peak areas of 40 predominant volatiles decreased as the ratio of TSP-to-chicken meat increased since TSP contained less volatiles than the meat. Over 12 days of storage, aerobic bacteria counts increased more in TSP-containing spreads. Redness (CIE a*) increased with higher chicken levels and decreased with higher TSP although panelists ratings of "likeness" of color did not differ among spreads. Sensory aroma "freshness" decreased for all spreads during storage. The results indicate that 50% or less of TSP does not affect most of the characteristics of chicken salad spreads.

Résumé

Des 'rillettes' ont été préparées à partir de viande de poulet cuite mélangée avec des protéines de soja texturées (PST), assaisonnées ou non, dans différents rapport (0-25-50-75-100%). La surface des pics des 40 principaux composés volatiles détectés par GC-MS diminue quand la proportion de PST augmente, probablement parce que ces dernières contiennent moins de composés volatiles que la viande. Après 12 jours de conservation, le développement des bactéries aérobies est plus prononcé dans les 'rillettes' contenant plus de PST. La couleur des 'rillettes' est plus rouge quand la proportion de viande augmente, mais le jury de dégustation n'exprime pas de préférence de couleur entre les différents mélanges proposés. L'arôme « Fraîcheur » décroît avec le temps de conservation quelque soit le mélange. Les résultats de cette étude indique que les 'rillettes' contenant 50% ou moins de PST ont des caractéristiques similaires à celles réalisées à base de viande uniquement.

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Introduction

Chicken salad has been generally defined as a refrigerated ready-to-eat food having a pH higher than 4.6, a water activity greater than 0.85, no secondary barrier to protect against temperature abuse and not receiving a thermal treatment prior to use to inactivate food-borne pathogens (NFPA, 1989). The chicken meat component of the salads contributes “meaty”, “chickeny”, or “chicken broth” flavor sensations although numerous factors affect cooked chicken meat flavor and volatile profiles (Ang, 1991). Textured soy protein concentrates have been processed to provide meat-like textures. Although flavor problems have been encountered with raw beans or soy flours due to astringent phenolic compounds (Seo and Morr, 1984), textured products are generally lower in undesirable qualities that are associated with odor and flavor (Warner et al., 1983). The objective of this study was to determine several quality characteristics of chicken salad spreads when varying levels of textured soy protein were used in combination with chicken meat and other typical ingredients normally used to manufacture chicken salads.

Materials and Methods

Dry textured soy protein (Central Soya) was hydrated with 2.2 parts by weight of 75 C water with and without 0.07% addition of natural chicken flavoring (Bell Flavors & Fragrances). Cooked deboned chicken breast and thigh meats in an approximate ratio of 1.5:1.0, respectively, was combined with TSP to provide 5 mixes of weight ratios ranging from 100% chicken to 0% chicken in 25% increments. Prior to combining, meat and TSP were separately chopped in a Food Processor (Cuisinart) to a small, fibrous particle size. The meat-TSP blends were held at 3-4 C for 1-4 h before use in preparation of the chicken salad spreads where other ingredients were added. Chicken salad spreads were made with 57.7% chicken-TSP mix, 31.7% mayonnaise, 5.8% water, 1.9% lemon juice, 1.7% dehydrated onion, and 1.2% dehydrated celery with the added water rehydrating the onion and celery. Spreads were held at 4 C for at least 24 h and then volatiles were determined using a GC-MS system consisting of a Hewlett-Packard 7694 Head-Space sampler interfaced with a HP 6890 Gas Chromatograph and a HP 5973 Mass Selective Detector. A 2.5 g sample of meat-TSP blend or salad spread was sealed in a 10 mL vial and heated at 90 C for 20 min after which the headspace volatiles of the sample were automatically injected into the head of a HP-5MS capillary column. The GC column temperature was set at 0C and raised at 10 C/min to 250 C with He as the carrier gas.

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For evaluation during storage, chicken salad spreads were vacuum-packaged in film (oxygen transmission rate of 7000 cc/m2/24 h) and held at 3 C for 12 days in 765 lux of lighting. Packages were approximately 15 cm x 13 cm and contained 200-205 g of spread. Packages from two replicates were removed from display as needed. Aerobic bacteria were determined at 0, 4, 8 and 12 days using a 25 g sample and sterile 0.1% peptone solution as diluent. After blending in a stomacher (Tekmar, Inc) for 1 min, serial dilutions were plated with standard Plate Count Agar (Difco) and plates incubated at 35 C for 48 h. Based on dilution, aerobic counts were expressed as colony forming units (CFU) per gram of sample. The pH values of spreads were determined at 0 and 12 days using a 10 g sample blended for 1 min with 100 mL distilled water. pH values were with an Orion pH meter (Orion) equipped with a combination electrode. A Minolta CR-300 Chroma Meter (Minolta Corporation) was used to measure redness (as CIE a*) of the chicken salad spreads. Measures were made directly though the packaging film. Two packages were used with 3 random fields selected per sample. A 12-member untrained sensory panel was used to evaluate chicken salad spread color "likeness" and aroma "freshness". In 3 replicate sessions, panelists marked evaluations on a 15 cm line scale having anchor words for aroma freshness of "stale/off-aroma" (0) to "fresh aroma" (15) and for likeness of color as "dislike color" to "like the color".

Results and discussion

The types of volatile compounds and their relative concentrations were expected to shift as the meat-TSP mixes changed from 100% chicken to 100% TSP and as other ingredients were added to the mixes to form chicken salad spreads. In mixes of 100% chicken meat, 35-36 of 40 volatiles were present whereas for 100% TSP, this decreased to 17-19 volatiles. Individual volatile peak areas also decreased. Flavoring addition to TSP added only 2 compounds, 5-methyl 3-heptene-2-one and 2-octanal, not found in unflavored meat-TSP mixes. After ingredient addition (mayonnaise, lemon juice, celery and onion) to meat-TSP flavored mixes, salad spreads analyzed only 24 dominant volatiles. Several new compounds were detected as a result of ingredient addition. Similar to volatiles of meat-TSP mixes, total volatile areas of chicken salad spreads decreased as TSP levels increased. Effects of less volatiles on flavor was not determined. It is simple to predict that higher levels of TSP use would dilute odor and flavor attributes of the spreads.

During storage, initial aerobic bacteria counts of chicken salad spreads ranged from 3.3-3.8 $\log_{10}$ CFU/g (Table 1) which approximates the level reported by Dawson et al. (1995) for fresh ground chicken meat. Spreads prepared with 25% and 50% TSP mixes had higher ($P<0.05$) counts after 12 days than other spreads. The spread prepared with 100% chicken meat had the lowest count (3.9 $\log_{10}$ CFU/g) at 12 days.

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This lower count may be related to pH of the 100% chicken mix, a factor previously noted as important in microbial outgrowth in meat-soy blends (Keeton and Melton, 1978). For days 0-8 there was not a 1 $\log_{10}$ CFU/g difference among the chicken salad spread samples. Initial pH of spreads ranged from 5.51-5.95 (Table 2) and increased with increase of TSP level. After 12 days of storage, spreads made with 50% and 100% TSP in the meat-TSP mix had a lower ($P<0.05$) pH than initial values. Lactic acid bacteria may have contributed to this decline since the spreads were vacuum-packaged with a moderate oxygen permeable packaging film. Instrumental color values using the CIE L*a*b* color space showed that initial 0-day redness (CIE a*) of chicken salad spreads was highest with higher contents of chicken meat and less red with increase of TSP content (Table 3). During 12 days of storage, spreads with more chicken decreased ($P<0.05$) in redness whereas those with more TSP increased in redness. Loss of redness relative to chicken meat concentration was likely due to forming a "tan-to-light brown" color characteristic of myoglobin oxidation. Increase ($P<0.05$) of redness observed in the spreads prepared with 75% and 100% TSP in the meat-TSP mixes cannot readily be explained. It is possible mineral oxidation within the composition of TSP may be the contributor to the redness increase noted.

Panel results for sensory evaluations of "likeness" of color and aroma "freshness" indicated no difference ($P<0.05$) among chicken salad spreads at any storage period (Table 4). For color, only the chicken salad spread made with a mix of 100% meat had color "likeness" deterioration during the 12 days of storage. This generally agrees with the CIE values found for "redness" of this spread. It is possible that the panelists recognized the degree of tan-to-light brown development as a loss of color in the 100% chicken containing spread as compared to the other TSP-containing spreads and rated it lower. Aroma "freshness" did not differ among the chicken salad spreads at any storage interval although time of storage decreased "freshness" ratings in all samples except the spread containing no chicken meat.

In summary, results for chicken meat-TSP mixes and for prepared chicken salad spreads showed shifts in various attributes. However, the results suggest TSP use at about 50% may provide attributes similar to spreads with only chicken meat.

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Table 1. Aerobic bacteria counts of chicken salad spreads during storage.

Storage time (days)	Aerobic Bacteria Counts (log CFU/g) Weight Percent of Textured Soy Protein					(SEM)
	0	25	50	75	100	
0	3.5 ^{bc,x}	3.8 ^{a,x}	3.5 ^{bc,y}	3.6 ^{b,x}	3.3 ^{bc,z}	(0.04)
4	3.7 ^{yw}	3.6 ^{w}	3.6 ^{y}	3.6 ^{w}	3.6 ^{y}	(0.05)
8	3.9 ^{ab,w}	4.2 ^{ab,w}	4.2 ^{a,x}	3.7 ^{b,w}	3.9 ^{ab,x}	(0.07)
12	3.9 ^{c,w}	5.5 ^{a,w}	5.9 ^{a,w}	4.8 ^{b,w}	4.8 ^{b,w}	(0.10)
(SEM)	(0.05)	(0.13)	(0.09)	(0.04)	(0.05)	

$^{a-c}$ Row means not having a common letter differ (P<0.05).
$^{w-z}$ Column means not having a common letter differ (P<0.05).

Table 2. pH values of chicken salad spreads at 0 and 12 days of storage.

Storage time (days)	pH of Chicken Salad Spread Weight Percent of Textured Soy Protein					(SEM)
	0	25	50	75	100	
0	5.51 ^{c}	5.76 ^{b}	5.89 ^{a,x}	5.92 ^{a}	5.95 ^{a,x}	(0.01)
12	5.43 ^{c}	5.75 ^{b}	5.89 ^{ab,y}	5.87 ^{a}	5.73 ^{b,y}	(0.02)
(SEM)	(0.02)	(0.01)	(0.01)	(0.01)	(0.05)	

$^{a-c}$ Row means not having a common letter differ (P<0.05).
$^{x-y}$ Column means not having a common letter differ (P<0.05).

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Table 3. Redness (CIE a*) of chicken salad spreads during storage.

Storage time (days)	Redness (CIE a*) Weight Percent of Textured Soy Protein					(SEM)
	0	25	50	75	100	
0	0.28 ^{b,w}	0.09 ^{b,w}	-0.06 ^{ab}	-0.09 ^{ab,y}	-0.04 ^{a,z}	(0.059)
4	-0.62 ^{d,wx}	-0.05 ^{d,x}	-0.28 ^{c}	-0.06 ^{b,xy}	0.32 ^{a,y}	(0.027)
8	-0.86 ^{d,x}	-0.70 ^{d,x}	-0.23 ^{c}	0.10 ^{b,x}	0.66 ^{a,x}	(0.022)
12	-1.53 ^{d,x}	-0.66 ^{c,x}	-0.09 ^{bc}	0.27 ^{ab,w}	0.87 ^{a,w}	(0.126)
(SEM)	(0.218)	(0.026)	(0.014)	(0.007)	(0.008)	

$^{a-d}$ Row means not having a common letter differ (P<0.05).

$^{w-z}$ Column means not having a common letter differ (P<0.05).

Table 4. Sensory evaluations of color and aroma during storage.

Storage time (days)	Sensory Attribute	Sensory Evaluation for Likeness of Color and Aroma Freshness Weight Percent of Textured Soy Protein					(SEM)
		0	25	50	75	100	
0	Color	11.2 ^{x}	11.4	11.6	11.5	11.1	(0.04)
4		10.9 ^{xy}	11.2	11.3	11.4	11.5	(0.04)
8		10.1 ^{xy}	11.3	12.0	13.1	13.6	(0.07)
12		9.7 ^{y}	10.5	10.6	10.9	10.4	(0.10)
(SEM)		(0.6)	(0.5)	(0.5)	(0.5)	(0.6)	
0	Aroma	12.1 ^{x}	12.2 ^{x}	12.1 ^{x}	11.8 ^{x}	11.3	(0.2)
4		11.6 ^{xy}	11.5 ^{xy}	11.9 ^{xy}	11.3 ^{xy}	10.8	(0.2)
8		10.8 ^{yz}	11.1 ^{y}	10.9 ^{yz}	10.9 ^{xy}	10.1	(0.2)
12		10.4 ^{z}	10.8 ^{y}	10.4 ^{z}	10.5 ^{y}	10.0	(0.2)
(SEM)		(0.4)	(0.4)	(0.5)	(0.6)	(0.8)	

$^{x-z}$ Column means within color or aroma not having a common letter differ (P<0.05).

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Session 5
Nutritional effects on meat and carcass quality

GROWTH CHARACTERISTICS AND PERFORMANCES OF MALE BROILERS

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Keywords : broiler, male, growth, meat quality, FA composition

Abstract

This research aimed at investigation of growth phases of male broilers and establishment of qualitative characteristics of carcasses, as well as of chemical content of breast muscles and thighs. The research was carried out on 45 male broilers of Ross 208 provenience, which were divided into 3 groups. Broilers were fed with starter and finisher diets containing 22.67%/20.43% of crude proteins and 14.19 MJ/kg/14.18 MJ/kg ME, respectively. After 42 days of fattening broilers gained weight of 2065.75 ±100.36 g. Asymmetric S-function was used for estimation of the growth phases, and parameters of the used model were the following: A=3.80 kg, b=1.42, c=2.70 and γ=0.18. Average weight of carcasses was 1815±25 g. Portions in the whole carcass were as follows: breast 33.75%, thighs with drumsticks 30.35%, wings 11.15% and loin 22.40%. Breast muscles differed statistically ($P<0.05$) than thigh muscles in the content of proteins (23.03% : 19.42%), fats (1.32% : 4.51%) and ashes (1.23% : 1.10%). Breast muscles contained more SFA (39.48% : 33.83%) and PUFA n-3 (6.02% : 3.71%), but less MUFA (29.61% : 35.63%) and PUFA n-6 (17.70% : 19.85%) when compared to the thigh muscles. Ratio PUFA n-6 / PUFA n-3 was better in the breast muscles than in the thighs.

Résumé

L'objectif de ce travail est d'étudier la courbe de croissance du poulet de chair mâle, d'établir les caractéristiques de qualité de la carcasse ainsi que la composition des

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muscles des cuisses et des filets. Cette étude a été réalisée sur 45 mâles Ross 208 répartis en 3 groupes. Les poulets ont été nourris avec des aliments démarrage et croissance renfermant respectivement 22.67 et 20.43% de protéines et 14.19 et 14.18 MJ/kg d'énergie métabolisable. Après 42 jours d'engraissement le poids des poulets a augmenté de 2065.75±100.36 g. Une fonction sigmoïde assymétrique a été utilisée pour estimer les phases de croissance. Les paramètres utilisés dans le modèle étaient les suivants : A=3.80 kg, b=1.42, c=2.70 et γ=0.18. Le poids moyen de la carcasse s'est élevé à 1815±25 g. Exprimés par rapport au poids de la carcasse, le pourcentage de muscles pectoraux s'est élevé à 33.75%, les cuisses et pilons à 30.35%, les ailes à 11.15% et le reste à 22.40%. Les muscles du filets diffèrent significativement des cuisses pour la teneur en protéines: 23.03% vs 19.42%, en lipides: 1.32% vs 4.51% et en cendres: 1.23% vs 1.10%. Ils renferment plus d'acides gras saturés : 39.48% vs 33.83% et d'acides gras poly insaturés n-3 :6.02% vs 3.71% mais moins d'acides gras mono insaturés : 29.61% vs 35.63% et de poly insaturés n-6 (17.70% vs 19.85% que les muscles des cuisses. Le rapport PUFA n-6 / PUFA n-3 est plus favorable dans les filet que dans les cuisses.

Introduction

Broilers of Ross provenience are usually used for fattening purposes in our country. Geneticists produce genotypes while having in mind some predefined goals, such as designing of specific products for specific markets, what requires production programmes for specific purposes. Kralik and Horvat (1992) and Kralik et al. (2001) published research results, which show that sex is a very important factor in phenotype traits of fattened broilers. Weight gain is approximately the same among male and female broilers during the first three weeks of fattening. Highly significant differences occur from 4^{th} to 6^{th} week of fattening for the benefit of male broilers. For that reason it is recommended to divide broilers according to their sex, and to compose diets with higher content of nutrients for male broilers. Večerek et al. (2002) carried out research on hybrid Ross 208 and 308 broilers, paying special attention to their usefulness. Over the 42-day-long fattening period, male Ross 208 broilers gained 2.50 kg of live weight, food conversion was 1.65 g/g, and the percentage of breast muscles in the total of live weight was 16.38%.

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Material and Methods

The research was carried out on 45 male broilers, which were divided into 3 replicates. From 1st to 21st day of fattening, broilers were fed with starter diet that contained 22.67% of crude protein and 14.19 MJ/kg ME. From 22nd to 42nd day of fattening, broilers were fed with finisher (pelleted) containing 20.43% of crude protein and 14.18 MJ/kg ME. PUFA n-6/PUFA n-3 ratio in starter was 8.73, and in finisher this ratio was 6.84. Broilers were kept on a straw bedded floor, feeding and drinking procedure was ad libitum.

Table 1. Nutrient composition of broiler diets (%)

Ration ingredient, %	Starter	Finisher
Maize	50.5	54.5
Soybean cake	31.3	30.3
Fish meal	8.0	5.0
Poultry fat	5.0	5.0
Limestone	2.0	2.0
Salt	0.2	0.3
Phosphonal (18% P)	1.3	1.7
Methionine	0.2	0.2
Premix[1]	1.0	1.0
Calculated contents		
Crude protein	22.67	20.43
Crude fat	7.89	7.86
Crude fibre	3.39	3.36
Lysine	1.41	1.22
Methionine + cystine	0.97	0.90
Calcium	1.16	1.03
Total phosphorus	0.77	0.76
Metabolizable energy, MJ/kg	14.19	14.18

[1] Supplemented per kg of feed:
- vitamins: A 15000 IU, D_3 2500 IU, E 30 mg, K_3 2.5 mg, B_1 1.5 mg, B_2 6.0 mg, B_6 4.0 mg, B_{12} 0.015 mg, pantothenic acid 15 mg, nicotinic acid 35 mg, folic acid 0.5 mg, holin chloride
- microminerals: Fe 30 mg, Cu 4.0 mg, Mn 80 mg, Zn 40 mg, Co 0.10 mg, Se 0.15 mg
- antioxidant 170 mg, diclavet 1.0 mg, lysine 1500 mg and methionine 1000 mg

Broilers' weight, food consumption and conversion were controlled once a week. Growth curve was described by asymmetric S-function. After 42 days of fattening and 10-hour period without feeding, broilers were slaughtered, and for the purposes of the research broiler carcasses were processed as "ready for grill". Carcasses were cut into main parts: breast, thighs with drumsticks, wings and loin. Abdominal adipose tissue was carefully removed from the carcasses and then weighed. The AOAC (1990)

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method was used for the analysis of basic chemical ingredients on 10 samples of breast and thigh muscles. Fatty acids content in the lipids of white and red chicken meat was found out by Chrompack CP-9000 chromatograph with flame ionisation detector. Fatty acids are shown in % of the total fatty acid value.

Results and Discussion

Table 2 and graph 1 show the average broiler weights obtained from the 1st day, or 1st week to the 6th week (y) and estimated weight f(x) at the respective age, as well as the food consumption, weight gain and food conversion.

Table 2. Growth parameters, consumption and food conversion

X (week)	Y (weight) (kg)	f(X) Estimated weight (kg)	Relative deviation (%)	Consumption (g) $\bar{x} \pm s$	Gain (g) $\bar{x} \pm s$	Conversion g/g of gain $\bar{x} \pm s$
0	0.033	0.035	0.01	-	-	-
1	0.151	0.116	0.03	129.10± 2.33	117.28± 2.45	1.10±0.02
2	0.371	0.348	0.05	358.05± 6.15	220.00± 4.55	1.63±0.04
3	0.751	0.776	0.14	667.03±21.08	380.33±19.04	1.76±0.05
4	1.303	1.357	0.51	1046.15±32.28	552.32±24.47	1.89±0.05
5	2.061	1.973	1.20	1504.40±58.20	757.38±40.18	1.99±0.07
6	2.479	2.516	0.64	1047.13±30.54	418.81±25.20	2.50±0.05
			Total	4751.00±20.25	2445.00±18.32	1.94±0.25

Function parameters: A=3.80; b=1.42; c=2.70; γ=0.18; F=0.014

Inflection point: (4.25; 1.52); X_B= (2.11; 0.39); X_C= (6.40; 2.70)

$$f(t) = \frac{A}{(1 + be^{-c\gamma t})^{1/\gamma}} \quad f_{(t)} = \frac{3.80}{(1 + 1.42 \cdot e^{-2.7 \cdot 0.18\, t})^{1/0.18}}$$

The Graph shows duration of the intensive growth from X_B to X_C.

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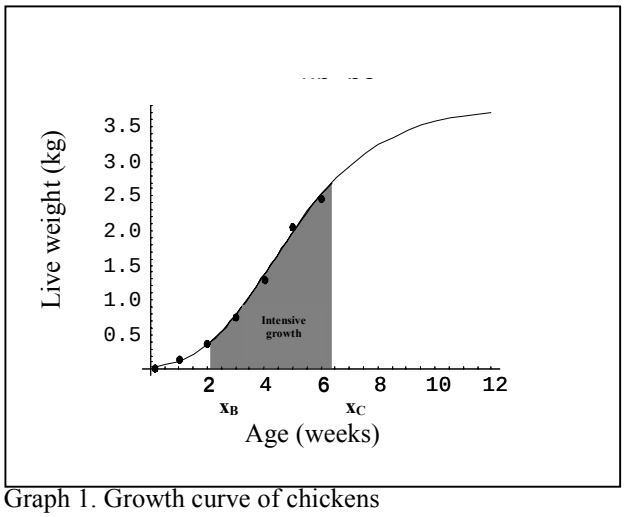

Graph 1. Growth curve of chickens

Portion of main parts in a carcass, and portion of tissue in breasts and thighs with drumsticks are shown in Tables 3 and 4. Tables 5 and 6 show chemical composition of meat, including the content of fatty acids.

Table 3. Indicators of carcass quality

Indicator	$\bar{x} \pm s$	Indicator	$\bar{x} \pm s$
Carcass weight, g	1815.05 ± 169.05	Wings, %	11.15 ± 0.96
Breast, %	33.75 ± 2.64	Loin, %	22.40 ± 2.62
Thighs with drumsticks, %	30.35 ± 2.09	Abdominal fat, %	2.32 ± 0.71

Table 4. Tissue portions in carcass parts and in whole carcass

Kind of tissue, %	Breast $\bar{x} \pm s$	Whole carcass $\bar{x} \pm s$	Thighs with drumsticks $\bar{x} \pm s$	Whole carcass $\bar{x} \pm s$
Muscular	76.66**±2.86	25.87**± 2.08	67.36 ± 2.36	20.45**±1.63
Fatty	9.86 ± 2.28	3.33 ± 0.83	12.26** ± 2.14	3.70 ± 0.71
Bones	13.47 ± 2.12	4.56 ± 0.81	20.38** ± 2.09	6.10**±1.18

**P<0.01

Table 5. Chemical composition of meat

Ingredient	Breast $\bar{x} \pm s$	Thighs $\bar{x} \pm s$
Water, %	74.43 ± 0.44	74.98 ± 0.50
Fat, %	1.32 ± 0.28	4.54** ± 0.77
Protein, %	23.03** ± 0.31	19.42 ± 0.40
Ash, %	1.23** ± 0.09	1.10 ± 0.02

**P<0.01

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Table 6. Fatty acids content in lipids of muscles

Fatty acid		Breast $\bar{x} \pm s$	Thighs $\bar{x} \pm s$
Lauric	C 12:0	0.47* ± 0.17	0.31 ± 0.13
Myristic	C 14:0	0.82 ± 0.12	1.05** ± 0.07
Pentadecaenoic	C 15:0	0.14 $^{n.s}$ ± 0.04	0.13 ± 0.05
Palmitic	C 16:0	23.43** ± 0.39	21.79 ± 0.41
Heptadecaenoic	C 17:0	0.30 $^{n.s}$ ± 0.04	0.30 ± 0.03
Stearic	C 18:0	13.88** ± 1.48	9.90 ± 1.06
Arachidic	C 20:0	0.14 $^{n.s}$ ± 0.01	0.16 ± 0.04
Behenic	C 22:0	0.18** ± 0.06	0.10 ± 0.03
Lignoceric	C 24:0	0.12* ± 0.04	0.09 ± 0.01
∑ SFA		39.48** ± 1.63	33.83 ± 1.31
Palmitoleic	C 16:1	1.52 ± 0.37	2.72** ± 0.42
Oleic	C 18:1	26.02 ± 2.37	31.54** ± 1.62
Eicosenoic	C 20:1	0.34 ± 0.05	0.47** ± 0.03
Nervonic	C 24:1	1.73** ± 0.40	0.46 ± 0.19
∑ MUFA		29.61 ± 2.37	35.63** ± 1.92
Linoleic	C 18:2n6	13.38 ± 1.19	17.23** ± 0.64
Eicosadienoic	C 20:2n6	0.67** ± 0.05	0.52 ± 0.04
Arachidonic	C 20:4n6	3.65** ± 0.80	2.10 ± 0.63
∑ PUFA n-6		17.70 ± 0.66	19.85** ± 0.38
α-linolenic	C 18:3n3	0.52 ± 0.10	0.82** ± 0.08
Eicosapentaenoic	C 20:5n3	0.62** ± 0.08	0.48 ± 0.05
Docosapentaenoic	C 22:5n3	1.91** ± 0.38	0.96 ± 0.26
Docosahexaenoic	C 22:6n3	2.95** ± 0.65	1.45 ± 0.42
∑ PUFA n-3		6.02** ± 0.90	3.71 ± 0.59
PUFA n-6 / PUFA n-3		2.95 ± 0.48	5.35** ± 0.81

*P<0.05; **P<0.01; $^{n.s.}$ P>0.05

With respect to the high portions of breasts (33.75%) and thighs with drumsticks (30.35%) in the carcasses, our research results prove that carcasses quality of Ross 208 male broilers is very high. Portion of breast muscular tissue in relation to the carcass weight was 25.87%, which was considerably higher than the same values published by Večerek et al. (2002), and yet approximately the same as the values established by Kralik et al. (1993) in their previous researches. According to the portions of particular tissues, such as fatty and muscular, and to the chemical composition, there is a difference observed among the main parts. Based on that, breasts consist of, statistically observed (P<0.01), more muscular tissue and less fatty tissue and bones when compared to thighs. Breast muscles also contain more proteins and ash and less fat than thigh muscles.

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Differences in the deposition of fatty acids into the lipids of muscular tissue were also observed. Lipids of breast muscles are richer in SFA, especially palmitic and stearic acids ($P<0.01$), and lipids of thigh muscles contain more MUFA (oleic acid) and PUFA n-6 (linoleic acid), $P<0.01$. Due to the fact that PUFA n-3 is highly recommended for human nutrition, especially for prevention of coronary diseases and stress induced consequences, it is important to emphasise that these fatty acids (EPA, DHA) are deposited in greater amounts in the lipids of breast muscles than in the lipids of thighs. Because of that reason, there is a better proportion of PUFA n-6 / PUFA n-3 in the breast muscles than in the thigh muscles.

Conclusion

Our research results show that Ross 208 broilers are suitable for production of high quality chicken meat. In broilers, breast meat contains more proteins and less fat. Furthermore, there is a better ratio of PUFA n-6 / PUFA n-3 in the lipids of breast muscles than in the lipids of thighs. Based on the conclusion that there are defined differences in the chemical composition of breast muscles and thigh muscles, there is also a difference in the nutritive quality of these two parts of the chicken meat.

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EFFECT OF DIETARY SUNFLOWER, LINSEED OR FISH OIL ON CHOLESTEROL AND FATTY ACID CONTENT IN TURKEY MEAT

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Keywords : turkeys, cholesterol, n-3 PUFA, arachidonic acid, vegetable oils

Abstract

Total cholesterol and 15 fatty acids were determined in breast meat (BM) and thigh meat (TM) of BUT Big 6 turkeys fed either a control diet (C) or diets enriched with sunflower oil (S), linseed oil (L) and fish oil (F), respectively. The L-diet as compared to the C-diet decreased ($P<0.05$) cholesterol content in BM of both males (40.7 vs. 57.3 mg/100 g) and females (51.9 vs. 57.3 mg/100 g), and in TM of males (71.1 vs. 84.4 - 85.5 mg/100 g). The ratio of n-6/n-3 PUFA (polyunsaturated fatty acids) in BM and TM of L-turkeys of both sexes (1.3 and 0.9 - 1.0, respectively) was not different ($P>0.05$) from corresponding tissues of F-groups. The content of very long chain n-3 PUFA was in BM and in TM of L-turkeys lower $P<0.05$) than in F-birds (67 vs. 116 and 151 vs. 254 mg/100 g).

Résumé

Le cholestérol total et les 15 acides gras ont été déterminés dans les filets (BM) et les cuisses (TM) de dindons BUT Big 6 nourris soit avec l'aliment contrôle (C) soit avec des aliments enrichis en huile de tournesol (S), de lin (L) ou de poisson (F) . L'aliment L comparé à l'aliment C a diminué la quantité de cholestérol dans les filets (BM) des mâles (40.7 vs 57.3 mg/100 g) et des femelles (51.9 vs 57.3 mg/100g) et aussi dans les cuisses (TM) des mâles (71.1 vs 84.4 – 85.5 mg/100g). Le ratio PUFA n-6/n-3 dans BM et TM des dindons des deux sexes recevant l'aliment Lin (1.3 et 0.9 – 1.0 respectivement) n´est pas différent de ceux du groupe F. Le contenu en en acides gras à très longue chaine de la série n-3 dans BM et TM des dindons du groupe L a été plus bas ($P<0.05$) que celui du groupe F (67 vs 116 and 151 vs 254 mg/100 g).

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Introduction

Despite the low fat content, cholesterol content in poultry meat is relatively high in comparison with other domestic animals (Engeseth and Gray, 1989), but on the other hand it is possible to manipulate it (Ajuyah et al., 1992). As far as fatty acids are concerned, the following topics are currently discussed from the viewpoint of human nutrition. First of all it is the ratio of n-6/n-3 PUFA, which should be as close to one as possible (Okuyama et al., 1997). This opinion follows from the different action of n-6 PUFA (proinflammatory) and n-3 PUFA (antiinflammatory) on immunity functions and inflammatory processes in animals and humans (Calder, 2001). Within n-3 PUFA, a much stronger physiological effect of very long chain (VLC n-3 PUFA; eicosapentaenoic, EPA and docosahexaenoic acid, DHA) types as compared to their precursor, α-linolenic acid has been suggested (Ollis et al., 1999). On the other hand, among n-6 PUFA, great attention is paid to arachidonic acid (AA), the most important metabolite of linoleic acid (Taber et al., 1998). Poultry meat can be an important source of AA in man (Li et al., 1998). The objective of the present study was to compare the effect of diets containing various plant oils with high proportion of either n-6 or n-3 polyunsaturated fatty acids to a diet with fish oil, on cholesterol content and fatty acid pattern in breast meat (BM) and thigh meat (TM) of turkeys.

Material and Methods

Forty males and forty females of the BUT Big 6 hybrid, respectively, were divided in four groups by ten birds within each sex and kept in cages. All birds were fed the control diet (C) until the 21st day of age. Thereafter, turkeys (both males and females) within a given group were fed either the control diet, or one of the three experimental diets, in which 4.72% was replaced by sunflower oil (S), linseed oil (L) and fish oil (F), respectively. Diets composition and their nutritive value, including fatty acid ratio is shown in Table 1. Turkeys were slaughtered at the age of 56 days and the following tissues were separated from each of eight birds within each group (males and females, respectively): musculus pectoralis profundus (breast meat, BM), and m. biceps femoris + m. semitendinosus + m. semimembranosus (thigh meat, TM). Extraction of total lipid from turkey meat (by hexane/2-propanol mixture), releasing of cholesterol from ester bonds and cholesterol determination by HPLC, and fatty acid determination by GC is described in detail in paper of Komprda et al. (2001).

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Results and discussion

Males fed the diet with linseed oil deposited significantly less ($P<0.05$) cholesterol in BM in comparison with the control and the F-group, and also tended ($P>0.05$) to deposit less cholesterol than S-males (Figure 1). Similar results were found also in BM of females. Lower ($P<0.05$) deposition of cholesterol in turkeys fed the diet with linseed oil in comparison with other groups was confirmed in TM of males as well. The lowering effect of the diet with high level of α-linolenic acid on cholesterol content in turkey tissues is possible to presume from Figure 1. Ajuyah et al. (1992) reported lower ($P<0.05$) cholesterol content in dark meat (but not in white meat) of spent hens fed the diet with 8 % or 16 % flax seed (PUFA n-3 content 31 and 40 % of total fatty acids, respectively) in comparison with the control diet (3 % of PUFA n-3), which is in accordance with our results regarding thigh meat of male turkeys.

Turkeys fed the diet with sunflower oil had the lowest percentage of saturated fatty acids (SFA). Both breast meat (31.4 and 30.6 %) and thigh meat (22.4 and 22.7 %) of both S-males and S-females, respectively, had lower ($P<0.05$) percentage of SFA not only than control birds (35.4 and 35.8 % in BM; 32.9 and 33.2 % in TM), but also than turkeys fed the diet with fish oil. On the other hand, both males and females fed the diet with fish oil had the highest ($P<0.05$) percentage of oleic acid (C18:1n-9) and total monounsaturated fatty acids (MUFA) both in BM (in the case of MUFA 25.1 and 24.5 %) and in TM (34.5 and 33.5 %, respectively). An opposite pole regarding MUFAs was the L-group. Both oleic acid percentage (17.4 and 16.7 % in males and females, respectively) and total MUFA percentage (18.4 and 17.9 %) in BM of linseed oil group was lower ($P<0.05$) than in BM of the control birds. As regards thigh meat of L-turkeys, both oleic acid percentage (19.8 % in males, 19.4 % in females) and MUFA percentage (21.7 % and 21.4 %) was also lower ($P<0.05$) in comparison with sunflower oil fed turkeys.

The ratio of n-6 and n-3 PUFA in human food should be as close as possible to one (Okuyama et al., 1997). From this viewpoint, meat of turkeys fed the diet with linseed oil or fish oil is an ideal food . Despite insignificant differences in each tissue ($P>0.05$) between L-and F-groups (both males and females), PUFAn-6/PUFAn-3 ratio of both F-groups (0.9) tended to be lower in comparison with L-groups (1.3) in BM, but higher in TM (1.4 vs. 0.9) in the present experiment. Similarly, Lopez-Ferrer et al. (1999) found a different n-6/n-3 PUFA ratio between groups of chickens fed the diet with either fish oil (0.58) or linseed oil (0.81). On the other hand, meat of turkeys fed the diet with sunflower oil (high content of linoleic acid), especially thigh meat, was very unfavorable from the above viewpoint in the present experiment (PUFAn-6/PUFAn-3 ratio 16 - 17).

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From the viewpoint of human nutrition, the most important metabolite of linoleic acid is arachidonic acid (AA; C20:4n-6). AA is a precursor of so called "proinflamatory" eicosanoids (Calder, 2001) and is therefore connected with autoimmune diseases, cardiovascular diseases, and some cancers (breast, prostate). From the above viewpoint, the most unfavorable product within the present experiment was meat of turkeys fed the diet with sunflower oil (54 – 76 mg AA/100 g). It was due to the fact, that the precursory linoleic acid constituted nearly 55 % of all measured fatty acids in the S-diet (Table 1). On the other hand, arachidonic acid content in both BM (22 – 25 mg/100 g) and TM (33 – 34 mg/100 g) of the linseed oil groups was not different ($P>0.05$) from fish oil fed turkeys. Both F- and L-birds had in average twice as low AA content in both tissues in comparison with other groups. Taber et al. (1998) found an AA content in (closer unspecified) turkey breast meat 59 mg/100 g.

As far as PUFAs of the n-3 family are concerned, birds from linseed oil group have, as expected, the highest percentage ($P<0.01$) of α-linolenic acid (LNA, C18:3n-3; 9.3 and 10.5 % in BM of males and females, and 24.2 and 25.3 % in TM of males and females, respectively) in both tissues in the present experiment. Turkeys fed the diet with fish oil did not differ ($P>0.05$) from the control group. However, also as expected, both F-males (18.5 in BM, 12.1 % in TM) and F-females (18.5 and 12.2 % in BM and TM, respectively) had the highest percentage ($P<0.01$) of the sum of EPA + DHA in both tissues.

According to Ollis et al. (1999), approximately 15 % of LNA is converted to VLC n-3 PUFA. Therefore, it is possible to calculate very long-chain fatty acid equivalent (VLCE), as follows: VLCE = 0.15 x LNA + (EPA + DHA). Content of n-3 PUFA in turkey breast and thigh meat, expressed by means of the VLC n-3 PUFA equivalent, is presented in Figure 2. Despite the same PUFA n-3 percentage in BM of L- and F-turkeys and substantially higher PUFA n-3 percentage in TM of L-group in comparison with F-group, VLCE of turkeys fed the diet with fish oil was in all cases higher ($P<0.05$).

It was concluded, that 5% dietary linseed oil was not sufficient to substitute fish oil as regards content of VLCE in turkey meat, but had the same (n-6/n-3 PUFA ratio, arachidonic acid content) or better (cholesterol content) effect on some other parameters of nutritive value of turkey BM and TM.

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Table 1. Composition of feed mixtures, including calculated energy content and measured nutrient and fatty acid content

	Feed mixture			
	C (control)	S (with sunflower oil)	L (with linseed oil)	F (with fish oil)
Ingredient (g/kg of fresh matter)				
Maize meal	346.5	330.2	330.2	330.2
Wheat meal	135.5	129.2	129.2	129.2
Soybean meal	316.8	301.9	301.9	301.9
Heat treated soybean	39.6	37.7	37.7	37.7
Fishmeal	49.5	47.2	47.2	47.2
Meat-and-bone meal	69.3	66.0	66.0	66.0
Ground limestone	6.9	6.6	6.6	6.6
Calcium phosphate	14.9	14.2	14.2	14.2
Sodium chloride	1.5	1.4	1.4	1.4
DL-methionine	0.9	0.8	0.8	0.8
L-lysine	0.7	0.7	0.7	0.7
Supplementary premix	7.9	7.5	7.5	7.5
Chromic oxide	9.9	9.4	9.4	9.4
Sunflower oil		47.2		
Linseed oil			47.2	
Fish oil				47.2
Nutrient content (g/kg of fresh matter)				
Crude protein	268.5	254.3	255.0	254.7
Crude fat	36.0	84.9	84.1	79.7
Metabolizable energy (MJ/kg of fresh matter)	11.8	13.0	12.9	12.9
Fatty acid (% of total fatty acids)				
C16:0	17.6	10.6	10.2	19.2
C18:0	5.7	4.7	4.6	4.9
C18:1n-9	32.3	26.1	20.7	30.0
C18:2n-6	37.5	54.7	25.3	19.6
C18:3n-3	1.9	1.1	36.5	1.3
C20:5n-3	0.7	0.8	0.5	3.9
C22:6n-3	1.0	0.4	0.6	3.8
n-6/n-3 ratio				

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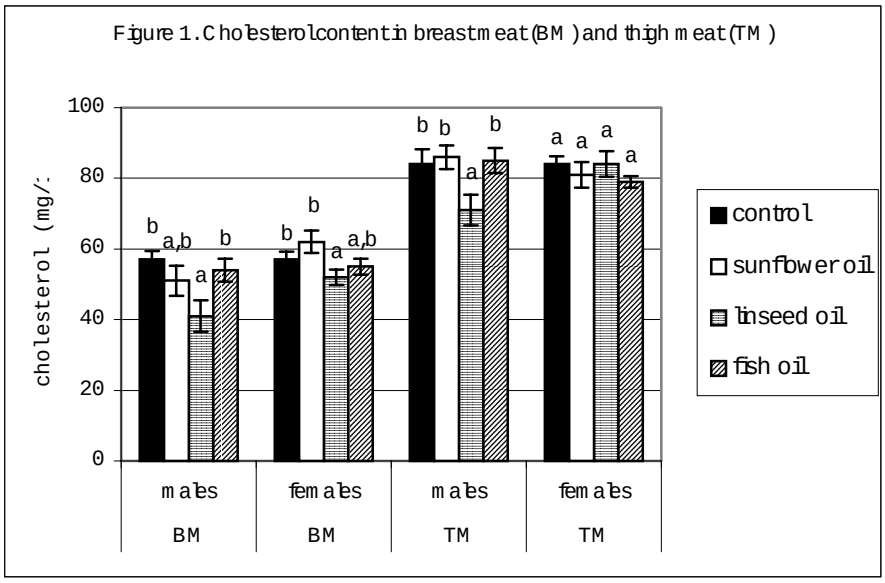
Figure 1. Cholesterol content in breast meat (BM) and thigh meat (TM)
cholesterol (mg/
100
80
60
40
20
0
males
BM
females
BM
males
TM
females
TM
control
sunflower oil
linseed oil
fish oil

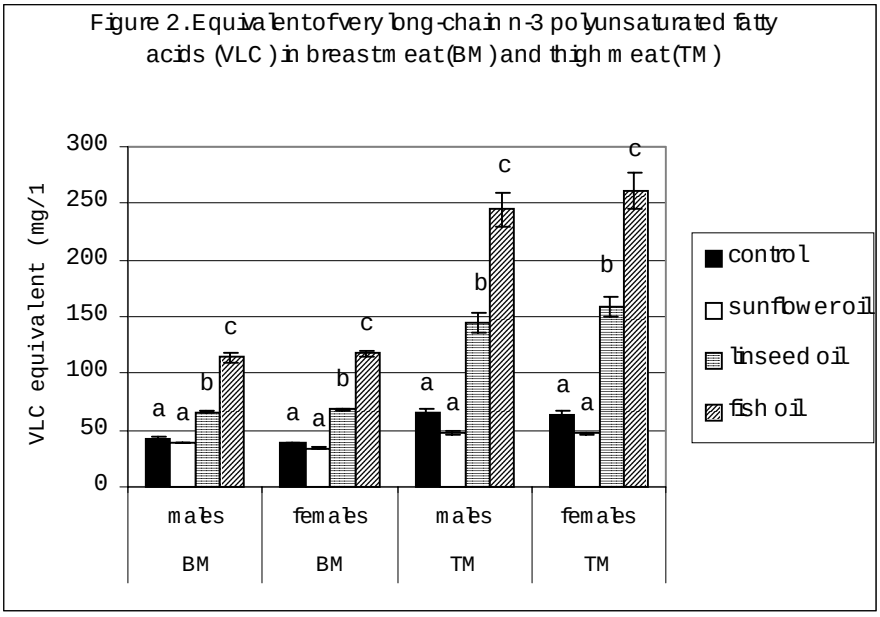
Figure 2. Equivalent of very long-chain n-3 polyunsaturated fatty acids (VLC) in breast meat (BM) and thigh meat (TM)
VLC equivalent (mg/1
300
250
200
150
100
50
0
males
BM
females
BM
males
TM
females
TM
control
sunflower oil
linseed oil
fish oil

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INTERACTION BETWEEN DIETARY UNSATURATION AND α–TOCOPHEROL LEVELS: VITAMIN E CONTENT IN THIGH MEAT

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Keywords : Dietary PUFA, α–Tocopherol, Thigh, Poultry.

Abstract

The present study was carried out to evaluate the influence of dietary polyunsaturated fatty acids (PUFA), at different levels of dietary supplementation with α–tocopheryl acetate (α–TA), on α–tocopherol (α–Toc) content in raw thigh meat. One hundred and ninety-two female broiler chickens were randomly distributed into 16 experimental treatments resulting from the combination of 4 levels of dietary polyunsaturated fatty acids (27, 38, 48 and 59 g/kg) and 4 levels of supplementation with α–TA (0, 100, 200 and 400 mg/kg). As is to be expected, dietary supplementation with α–TA increased α–Toc content of thigh meat. But the vitamin E enrichment magnitude in meat depends on the dietary PUFA content ($P \leq 0.001$). Furthermore, α–Toc content of thigh meat was reduced as the inclusion of dietary PUFA increased. Thus, in treatments supplemented with 100 mg α–TA/kg, an increase of 32 g dietary PUFA significantly reduced α–Toc content of meat by 52%.

Résumé

Cette expérience a été conduite pour évaluer l'influence des acides gras polyinsaturés alimentaires (PUFA), avec différents niveaux d'acétate de α-tocophérol (α-TA), sur le contenu de α-tocophérol (α-Toc) de la viande crue de la cuisse. Cent quatre-vingt douze femelles de poulet de chair ont été réparties au hasard dans 16 traitements expérimentaux correspondant à 4 niveaux d'acides grasses polyinsaturés alimentaires (27, 38, 48 et 59 g/kg) et 4 niveaux de supplémentation en α-TA (0, 100, 200 et 400 mg/kg). La supplémentation du régime en α-TA augmente le contenu de α-Toc de la viande de la cuisse. Mais l'importance de l'enrichissement en vitamine E dans la viande dépend du contenu des PUFA dans la diète ($P< 0.001$). De plus, le contenu en α-Toc dans la viande de la cuisse est diminué quand l'apport des PUFA dans la diète

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augmente. Ainsi, dans les traitements supplémentés avec 100 mg α-TA/kg, une augmentation de 32 g des PUFA dans le régime réduit le contenu de α-Toc de la viande de 52%.

Introduction

There is a trend towards rising unsaturation degree in chicken meat for a double reason. On the one hand, human nutritive recommendations suggest reducing saturated fatty acids intake, and on the other hand, the use of animal fats has been reduced in Europe. The higher unsaturation degree of meat produces organoleptic detriment (Bou et al., 2001) and leads to a major susceptibility to lipid oxidation (Grau et al., 2001). It is well established that dietary α–Toc supplementation prevents lipid oxidation (De Winne and Dirinck, 1996; Grau et al., 2001) and increases vitamin E content in poultry meat (Grau et al., 2001). Nevertheless, there are few data regarding the balance between dietary PUFA and vitamin E to reach nutritional benefits in meat.

Material and Methods

Animals and diets

One hundred and ninety-two female broiler chickens at one day of age were randomly distributed into 16 dietary treatments of three replicates each. Broilers were kindly supplied by Terra-Avant S.A. (Girona, Spain). The animals were housed in groups of four in forty-eight cages under standard conditions of temperature, humidity and ventilation.

The diets were formulated according to exceed the requirements recommended by the NRC (1994) on the basis of maize and soya. The experimental treatments were resulting from the combination of 4 levels of dietary polyunsaturated fatty acids: 27, 38, 48 and 59 g/kg and 4 levels of supplementation with α-TA: 0, 100, 200 and 400 mg/kg. The unsaturation degree was achieved by adding increasing proportions (2, 4, 6 and 8%) of a linseed and fish oil mixture (ratio 4:1) (Table 1).

Feed and water were provided ad libitum during the 40 days of experimental period. Feed samples were taken three times during the experiment for α-Toc content determination.

Sample collection

At the end of the experimental period, two animals per cage were killed in a commercial slaughterhouse. Thigh muscles were removed and weighed individually.

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Thighs were boned and ground with skin. Samples were freeze-dried, ground and stored at –20°C until further analyses.

α–Tocopherol analysis

α-Toc from feeds and thigh was extracted as described previously by Jensen et al. (1999) starting from 2 g of feeds and 100 mg of freeze-dried sample.

Table 1. Composition of experimental diets.

Ingredients (%)	**Dietary unsaturation (g/kg)[4]**			
	27	**38**	**48**	**59**
Maize	58.49	52.68	46.86	41.02
Soya 48	35.49	36.42	37.34	38.24
Added oil[1]	2.00	4.00	6.00	8.00
Almond husk	0.00	2.92	5.84	8.76
Dicalcium phosphate	1.75	1.76	1.76	1.77
Calcium carbonate	1.08	1.07	1.06	1.05
Salt	0.57	0.57	0.57	0.57
Vitamin mineral mix[2]	0.40	0.40	0.40	0.40
DL-Methionine	0.18	0.19	0.19	0.20
L-Lysine	0.05	0.03	0.02	0.00
Chemical analysis (%)				
Dry matter	88.26	89.08	89.42	90.28
Crude protein	21.31	21.82	21.48	21.85
Crude fat	4.45	6.30	8.40	9.91
Ash	5.79	6.10	5.85	6.20
ME (Kcal/kg)[3]	3000	3003	3006	3007

[1] Linseed and fish oil mixture in a ratio 4:1.

[2]Vitamin and mineral mix per kg of feed: Vitamin A: 12000 UI; Vitamin D_3: 2400 UI; Vitamin K_3: 3 mg; Vitamin B_1:2.2 mg; Vitamin B_2: 8 mg; Vitamin B_6: 5 mg; Vitamin B_{12}: 11 µg; Folic acid: 1.5 mg; Biotin: 150 µg; Calcium pantotenate: 25 mg; Nicotinic acid : 65 mg; Mn: 60 mg; Zn: 40 mg; I: 0.33 mg; Fe: 80 mg; Cu: 8 mg; Se: 0.15 mg.

[3]Estimated values.

[4]Each level of unsaturation was supplemented with four differents levels of α–TA (0, 100, 200 and 400 mg/kg).

Statistics

ANOVA was carried out to determine whether the factors studied (dietary unsaturation and α-TA level) affected α-Toc content in thigh meat. In all cases, $P \leq 0.05$ was considered significant.

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Results and discussion

The α-Toc content of raw thigh, expressed as mg/kg of thigh is shown in Table 2. Dietary supplementation with α-TA and dietary unsaturation level had significant effect ($P \leq 0.0001$) on the α-Toc content in thigh.
α-Toc content of thigh significantly increased with the dietary supplementation with α-TA. Several authors have reported that α–Toc content in poultry tissues increases linearly with dietary supplementation (Jensen et al., 1999; Cortinas et al., 2001). However, there is a wide range of variability in the α–Toc content of chicken meat obtained by different authors with similar levels of α–TA supplementation. This variation can be due in part to the studied tissue, and mainly to the lipid composition of the meat, depending on amount and profile.

Table 2. Effect of dietary unsaturation and α-tocopheryl acetate supplementation on α–tocopherol content in thigh meat (expressed as mg/kg thigh)[1].

Dietary PUFA (mg/kg)	**Global Mean**	**α–Tocopheryl Acetate (mg/kg)**			
		0	**100**	**200**	**400**
27	23.17^{x}	0.33^{g}	12.57^{ef}	24.34^{cd}	55.46^{a}
38	18.48^{y}	0.43^{g}	12.89^{ef}	19.05^{de}	41.56^{b}
48	14.63^{z}	0.10^{g}	10.34^{ef}	17.57^{de}	30.52^{c}
59	12.93^{z}	0.04^{g}	6.05^{fg}	14.98^{ef}	30.64^{c}
Global Mean		0.22^{z}	10.46^{y}	18.98^{x}	39.55^{w}
SEM	1.840				
P values					
Unsaturation		0.0001			
α-TA		0.0001			
Unsaturation × α-TA		0.0001			

[1]Values given in this table correspondence to least-square means obtained from ANOVA (n=6) and their pooled SEM. α-TA: α-Tocopheryl acetate.
[a,b,c,d,e,f,g]: Different superscripts indicate significant differences in the interaction between dietary unsaturation and α-tocopheryl acetate supplementation.
[w,x,y,z]: Different superscripts indicate significant differences in the global means.

At a dietary levels of 200 mg α–TA/kg approx., Grau et al. (2001) found higher α–Toc content in thighs with skin (from 21.8 to 25.2 mg α–Toc/kg) than those (De Winne and Dirinck, 1996; Maraschiello et al., 1999; Ruiz et al., 1999) in thighs without skin and therefore lower lipid content (from 8.7 to 17.5 mg α–Toc/kg).

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But probably the more important factor which causes the main differences among works are those related to the PUFA composition of the diet. The present work shows that dietary unsaturation affected the α-Toc deposit in thigh. As such, α-Toc content of thigh was reduced as the inclusion of dietary PUFA increased. Thus, an increase of 32 g dietary PUFA significantly reduced α-Toc content of meat by 52, 38 and 45% in treatments supplemented with 100, 200 and 400 mg α-TA/kg, respectively ($P \leq 0.05$). In literature studies which use fat and oil sources varying in the unsaturation degreee found variable results concerning α–Toc accumulation in chicken meat.

Dietary unsaturation gives rise to higher content of PUFA in chicken meat, which has a higher oxidative status (Grau et al., 2001). The relative quantities of α–Toc required to protect fatty acid are higher as double bounds in the molecule of the fatty acid increase (Witting and Horwitt, 1964). For that reason, studies adding to diets fish oil rich in long chain PUFA with a high number of double bounds found a reduction in the α–Toc content in chicken tissues (Miller and Huang, 1993; Husveth et al., 2000; Surai and Sparks, 2000; Cortinas et al., 2001). However, authors who used dietary vegetable oils with high levels of PUFA with a lower number of double bounds, did not find differences in α-Toc content in chicken tissues (Cherian et al., 1996; Maraschiello et al., 1999; Ruiz et al., 1999; Grau et al., 2001).

In conclusion, it is important to know the amount of PUFA, as well as the degree of unsaturation of PUFA of diets to optimise and to adjust the amount of α-TA supplemented in chicken feeds.

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OIL SOURCES AND VITAMIN E LEVELS IN THE DIET ON THE COMPOSITION OF FATTY ACIDS IN ROOSTERS MEAT

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Keywords: roosters, carcass, vitamin E, fatty acids

Abstract

Two hundred and forty 30-week-old White Leghorn roosters were distributed in a completely randomized factorial arrangement of 5 X 2, using five oil sources (sunflower, soybean, canola, linseed and fish) and two levels of antioxidants (30 and 400 mg of vitamin E/kg). The aim of this study was to evaluate the effect of the ω6:ω3 ratio, by including different oil sources and also of supplementing the diet with vitamin E on the meat composition. In the chest meat, the use of fish oil in the diet increased ($P<0.05$) the total content of the ω3 fatty acids as compared to other oil sources, specifically the contents of C20:5ω3 and C22:6ω3. The offering of linseed oil in the diet resulted in a larger total content of ω3 fatty acids and a reduction ($P<0.05$) in C18:2ω6 and in the ratio of ω6:ω3 as compared to soybean and sunflower oils.

Résumé

Deux cents quarante coqs de la race blanche Leghorn, âgés de 30 semaines, ont été répartis au hasard en 5x2 lots correspondant à cinq sources d'huile (soja, cânola, tournesol, lin et poisson) et deux niveaux de vitamine E (30 et 400 mg/kg dans l'aliment). L´objectif de cette étude était de comparer l'apport de différentes sources de matières grasses et de niveaux de vitamine E sur les dépôts des acides gras dans la viande des coqs. L´introduction d'huile de poisson et d'huile de cânola dans la nourriture a réduit le contenu de C18:2ω6 et C20:4ω6 ($P<0.05$), avec pour

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conséquence la diminution du rapport ω6:ω3. L'apport d'huile de poisson a augmenté ($P<0.05$) le contenu de C22:6ω3 dans la cuisse. L'introduction d'huile de lin dans l'aliment a induit un contenu plus élevé en acides gras ω3 et réduit également le rapport ω6:ω3. Les huiles de soja et de tournesol ont augmenté la teneur en acides gras ω6 et reduit ceux des ω3 dans la viande des cuisses des coqs.

Introduction

Polyunsaturated fatty acids of the ω3 and ω6 series are known to be essential nutrients in diet. Because of the competition between ω6 and ω3 PUFAs for enzymes involved in fatty acid elongation, desaturation and incorporation into tissue lipids, it has been recommended for human diet the ratio of ω6:ω3 between 4:1 and 10:1 (British Nutrition Foundation, 1992). Would be useful for consumers an alternative means of providing a dietary supply of balanced fats which reflect the nutricional guideline and/or targeted minimum nutrient requirement. The practical method to manipulate the composition of fatty acid in meat product, for improved nutritional value, is by modification of the animal diet. In association with this recommendation, special emphasis has been given to the antioxidants. The aim of this study was to evaluate the effect of the ω6:ω3 fatty acid ratio, by including different oil sources and also of supplementing the diet with vitamin E on the meat composition.

Material and Methods

Two hundred and forty 30-week-old White Leghorn roosters were housed in individual cages in a completely randomized factorial arrangement of 5 X 2, using five oil sources (sunflower, soybean, canola, linseed and fish) and two levels of antioxidant (30 and 400 mg of vitamin E/kg). The antioxidant used in this study was Lutavit E 50® (alpha-tocopherol acetate, BASF Corporation). Six percent oil was added regardless of the source and the total fat content of the experimental diets was 8% (isolipidic). The basis of all the diets was a standard commercial mash for breeders. The diet composition was based on the nutritional requirement according to NRC (1994). The principal fatty acids found in the diets can be observed in Table 1.
In the 54th week of age, the roosters were slaughtered, 4 birds/treatment being removed, totaling 40 chest and thigh samples each for analysis with respect to their fatty acid compositions. The lipids contained in the samples were extracted using the technique of Folch et al. (1957) and the composition of fatty acid were determined by gas liquid chromatography (Firestone, 1998). The samples were subjected to

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transmethylation with a base using the methodology of Hartman and Lago (1973), and the fatty acids were converted to methyl esters and identified by comparing the retention times with the standard (Sigma and Polyscience, U.S.A.). Quantification was done by normalization (area %). In the case of the poultry meat the results were expressed in mg/100g of edible portion, using the lipid conversion factor (F) reported by Holland et al. (1994). The data was assessed by analysis of variance using the System for Statistical and Genetic Analyses (UFV, 1997). Significant differences were determined by SNK test with a 5% level of probability. When there was an interaction ($P<0.05$), the effect of the vitamin E level inside each oil source was determined.

Results and Discussion

The diet containig fish oil reduced ($P<0.05$) the total content of ω6 fatty acid in the thigh meat as compared to soybean, sunflower and linseed oils (Table 2), specifically the content of C18:2ω6. This information corroborates previous studies (Cherian et al., 1996). There was also a reduction ($P<0.05$) in C18:3ω3 in the thigh meat, although this was justified as a function of the conversion of the C18:3ω3 in DHA (docosahexaenoic) and consequent reduction of the ratio of ω6:ω3 fatty acid as compared to soybean and sunflower oils. The use of fish oil on the total content of ω6 fatty acid is linked to the ratio of ω6:ω3 in the diet, which provided a greater deposition of ω3 fatty acid at the expense of the ω6 fatty acid. In the chest meat, the use of fish oil in the diet increased ($P<0.05$) the total content of the ω3 fatty acids as compared to other oil sources, specifically the contents of C20:5ω3 and C22:6ω3. An interaction effect was verified between the vitamin E level and the fish oil ($P<0.05$) increasing the total content of C20:5ω3 and C22:6ω3, consequently increasing the total content of ω3 fatty acids acids in the chest meat. This suggests that the vitamin E supply in the fish-oil-based diet decreased the oxidation of the ω3 fatty acids, resulting in greater deposition in the carcass, which corroborates previous studies (Cherian et al. 1996). The canola-oil-based diet reduced ($P<0.05$) the total content of ω6 fatty acids in the thigh meat as compared to other oil sources, such as C18:2ω6, resulting in a decrease in the ratio of ω6:ω3 as compared to soybean and sunflower oils. There was also a reduction ($P<0.05$) in total content of ω3 fatty acids as compared to fish and linseed oils. Despite the decrease in the total content of ω6 fatty acids in these roosters, the ratio of ω6:ω3 in the carcass was still within the guideline recommended for man from 4:1 to 10:1 (British Nutrition Foundation, 1992).

The offering of linseed oil in the diet resulted in a larger total content of ω3 fatty acids ($P<0.05$), more precisely of C18:3ω3, and a significant reduction in C18:2ω6 and in the ratio of ω6:ω3 as compared to soybean and sunflower oils. These data corroborate

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former studies (Olomu and Baracos, 1991). The diet containing linseed oil showed a high PUFAs content (60.9%), mainly C18:3ω3 (32.3%), and a reduced ratio of ω6:ω3 of 0.88 (Table 1), that propitiated a high PUFAs percentage, as for instance, that of C18:3ω3 in thigh meat as compared to other oil sources. The significant reduction in C18:2ω6 and in the ratio of ω6:ω3 fatty acids in the thigh meat of the roosters fed on the diet containing linseed oil, is probably related to their high content in ω3 fatty acids, which might have induced the suppression of the metabolism of the ω6 series. The use of soybean and sunflower oils resulted in higher ($P<0.05$) levels of C18:2ω6 in both cases and of C20:4ω6 specifically for the sunflower-oil-based diet. Both oils significantly reduced the level of ω3 fatty acids in the thigh as compared to linseed and fish oils and consequently provided increase in the ratios of ω6:ω3 fatty acids in the thigh as compared to canola, linseed and fish oils. This information corroborates previous studies (Scaife et al., 1995). Sunflower and soybean oils contain a lot of PUFAs, 68.4% and 59.9% respectively, and of these totals, 68.3% and 55.4% were constituted by ω6 fatty acid (Table 1). Thus, this supply influenced the level of ω6 fatty acids in the thigh meat.

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Tables

Table 1 - Profile of fatty acids found in the experimental diets

	Diets with oils of				
Fatty Acids (Area %)	Soybean	Sunflower	Canola	Fish	Linseed
Saturated	16.2	10.2	10.5	17.5	12.7
Monounsaturated	28.1	26.2	55.2	30.8	26.4
Polyunsaturated	55.7	63.6	34.3	51.7	60.9
omega-6 (ω6)	51.9	63.1	29.8	46.1	28.6
omega-3 (ω3)	3.8	0.5	4.5	5.5	32.3
Ratio of ω6:ω3	13.6	126.2	6.62	8.38	0.88

Table 2 – Content of the series ω6 and ω3 fatty acid in the thigh and chest meat [1]

Oil Sources	**Vitamin E levels (mg/kg of diet)**						
	30	400	$\bar{x}$ Oil		30	400	$\bar{x}$ Oil
	THIGH MEAT				CHEST MEAT		
	C18:2ω6 – LINOLEIC ACID (mg per 100g)						
Soybean	615.5	598.1	606.8 [a]	Soybean	156.8	217.3	187.1
Canola	172.6	55.2	113.9 [d]	Canola	105.1	137.8	121.4
Sunflower	542.6	633.9	588.3 [a]	Sunflower	150.1	197.2	173.7
Linseed [2]	592.9	373.3	483.1 [b]	Linseed	157.3	123.2	140.2
Fish	322.5	349.3	335.9 [c]	Fish	174.3	216.5	195.4
$\bar{x}$ Vitamin E	449.2	402.0	425.6		148.7	178.4	163.5
Standard Error		47.15				34.59	
	C20:4ω6 – ARACHIDONIC ACID (mg per 100g)						
Soybean [2]	98.4	70.7	84.6 [b]	Soybean	83.6	93.2	88.4 [a]
Canola [2]	46.0	81.6	63.8 [c]	Canola [2]	61.8	40.5	51.2 [c]
Sunflower [2]	89.0	125.6	107.3 [a]	Sunflower	78.5	82.9	80.7 [ab]
Linseed	102.3	90.4	96.4 [ab]	Linseed [2]	86.1	55.6	70.8 [b]
Fish	76.2	76.5	76.4 [bc]	Fish [2]	65.9	114.8	90.4 [a]
$\bar{x}$ Vitamin E	82.4	89.0	85.7		75.2	77.4	76.3
Standard Error		8.68				6.10	
	TOTAL OF OMEGA-6 FATTY ACID (mg per 100g)						
Soybean	744.4	704.7	724.6 [a]	Soybean	247.3	315.9	281.6 [a]
Canola	225.4	114.4	169.9 [c]	Canola	171.2	156.9	164.1 [b]
Sunflower	648.6	776.6	712.6 [a]	Sunflower	243.6	290.8	267.2 [a]
Linseed	643.2	470.5	556.8 [a]	Linseed	230.7	179.8	205.3 [ab]
Fish	402.9	426.2	414.5 [b]	Fish	240.2	332.9	286.6 [a]
$\bar{x}$ Vitamin E	532.9	498.5	515.7		226.6	255.3	240.9
Standard Error		69.04				30.79	
	C18:3ω3 - ALPHA-LINOLENIC ACID (mg per 100g)						
Soybean	22.9	19.2	21.0 [b]	Soybean	4.0	8.2	6.1 [b]
Canola	11.5	2.8	7.2 [c]	Canola	2.9	4.0	3.4 [b]

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Sunflower	4.6	4.4	4.5 [c]	Sunflower	2.7	3.3	3.0 [b]
Linseed [2]	83.7	107.7	95.7 [a]	Linseed	24.5	28.3	26.4 [a]
Fish	11.1	9.4	10.3 [c]	Fish	3.6	8.2	5.9 [b]
$\bar{x}$ Vitamin E	26..8	28.7	27.7		7.58[B]	10.4[A]	9.0
Standard Error		3.68				1.55	
	C20:5ω3 - EICOSAPENTANOIC ACID (mg per 100g)						
Soybean	7.8	7.5	7.6 [a]	Soybean	0.6	1.1	0.9 [c]
Canola	1.7	1.8	1.7 [b]	Canola [2]	1.5	5.8	3.6 [a]
Sunflower	7.3	5.7	6.5 [a]	Sunflower	0.8	0.5	0.6 [c]
Linseed	10.5	8.8	9.7 [a]	Linseed [2]	3.0	1.8	2.4 [b]
Fish	10.1	9.2	9.7 [a]	Fish [2]	2.7	5.7	4.2 [a]
$\bar{x}$ Vitamin E	7.5	6.6	7.1		1.7	3.0	2.3
Standard Error		1.08				0.36	
	C22:6ω3 - DOCOSAHEXANOIC ACID (mg per 100g)						
Soybean	9.1	8.5	8.8 [b]	Soybean	10.2	8.1	9.1 [b]
Canola	12.8	14.1	13.4 [b]	Canola	8.1	10.9	9.5 [b]
Sunflower	5.9	12.0	8.9 [b]	Sunflower	2.8	4.0	3.4 [c]
Linseed	14.7	17.1	15.7 [b]	Linseed	6.7	12.6	9.6 [b]
Fish	37.3	28.0	32.7 [a]	Fish[2]	27.4	72.2	49.8 [a]
$\bar{x}$ Vitamin E	16.0	15.9	16.0		11.0	21.6	16.3
Standard Error		2.53				2.11	
	TOTAL OF OMEGA-3 FATTY ACID (mg per 100g)						
Soybean	53.5	36.8	45.1 [c]	Soybean	24.6	26.2	25.4 [c]
Canola	30.9	35.6	32.9 [c]	Canola	25.2	18.1	21.6 [c]
Sunflower	21.2	24.2	22.7 [c]	Sunflower	12.6	11.9	12.2 [d]
Linseed	117.3	144.6	131.0 [a]	Linseed [2]	52.1	61.4	56.7 [b]
Fish	72.0	58.3	65.1 [b]	Fish [2]	38.0	106.4	72.2 [a]
$\bar{x}$ Vitamin E	59.0	59.9	59.48		30.5	44.8	37..6
Standard Error		9.31				2.93	
	RATIO OF ω6:ω3						
Soybean	14.0	19.8	16.9 [b]	Soybean	10.37	16.94	13.66 [b]
Canola	7.0	3.6	5.3 [c]	Canola	9.64	10.76	10.20 [b]
Sunflower	30.7	31.1	30.9 [a]	Sunflower	29.12	25.00	27.06 [a]
Linseed	4.1	3.3	3.7 [c]	Linseed	5.34	2.97	4.15 [c]
Fish	5.6	7.9	6.8 [c]	Fish	7.64	3.14	5.39 [c]
$\bar{x}$ Vitamin E	12.3	13.2	12.7		12.4	11.7	12.1
Standard Error		1.77				1.98	

[a, b, c, d] Averages values within the same column with no common superscript differ significantly by the test Student Newman Keuls (P<0.05)

[A, B] Averages values within the same line with no common superscript differ significantly by the F test (P<0.05)

[1]/Values are means of four repetitions per treatment.

[2]/Significant effect (P<0.05)

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THE POTENTIAL IMPACT OF 3-HYDROXY-3-METHYLBUTYRATE (HMB) FED IN THE WATER ON CARCASS QUALITY AND MEAT LIPID PEROXIDATION IN YOUNG BROILERS.

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Keywords : carcass quality, HMB, cholesterol, lipid peroxidation, TBARS

Abstract

3-hydroxy-3-methylbutyrate (HMB) has been shown in several studies to reduce broiler mortality by improving immune system function in young chicks. Present studies were conducted to evaluate carcass quality of young broilers fed standard diet and receiving CaHMB dissolved in drinking water. At 49 d of age birds were killed and fresh muscles from breast and leg were analyzed for tissues protein, dry matter, fat and cholesterol content. The remainders of muscles were stored for 1 month in -18^0C and then the content of thiobarbituric acid-reactive substances (TBARS) was determined. Muscle cholesterol was significantly lower in HMB-treated birds when compared to controls. TBARS decreased in muscles of birds receiving HMB. In conclusion, our studies show that HMB lowers muscle cholesterol content and inhibits lipid peroxidation in muscles of young broilers.

Resume

Plusieurs études ont montré que le 3-hydroxy-3-methylbutyrate (HMB) réduit la mortalité des poulets en améliorant leur statut immunitaire. Cette étude a eu pour but d'évaluer la qualité des carcasses des poulets recevant un régime standard et du CaHMB dans l'eau de boisson. A l'age de 49 jours, les oiseaux ont été abattus et le tissu musculaire frais du filet et des cuisses a été analysé pour déteminer leur teneur en proteines, en matière sèche, en graisses et en cholesterol. Le reste du tissu a été conservé pendant un mois a -18^{o}C en vue du dosage des TBARS (thiobarbituric acid réactive substances). Nos résultats montrent que la teneur du muscle en cholesterol et

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TBARS était significativement plus faible chez les poulets supplementés en HMB. En conclusion, le HMB par son effet sur le métabolisme du cholestérol et la peroxidation lipidique pourrait avoir un impact intéressant sur la qualité des carcasses.

Introduction

3-hydroxy-3-methylbutyrate (HMB), a catabolite of leucine is metabolized from 2-ketoiso-caproate (KIC), the keto acid of leucine, in the liver by KIC-dioxygenase (Saburin and Bieber 1981). HMB is present in both plant and animal foods and is currently available as a nutritional supplement. The theory behind HMB supplementation is that HMB is metabolized to 3-hydroxy-3-methylglutaryl CoA (HMG-CoA), which is used for cholesterol synthesis (Nissen and Abumrad, 1997). HMG-CoA can be a rate limiting substrate when cholesterol synthesis is in great demand, such as during periods of rapid cell growth or membrane repair. Thus, HMB may provide the necessary amount of HMG-CoA for cholesterol synthesis and subsequent membrane production during periods of high muscular stress. Preliminary studies indicate that HMB ingestion may enhance strength gains, decrease low-density lipoprotein (LDL) concentrations, decrease muscle damage and increase lean body mass in humans (Panton et al. 2000). Extensive animal research has been conducted analyzing the safety of HMB supplementation. No adverse effects have been reported in animals consuming between 8 and 5000 mg.kg.d for a period of 1-16 wk. In broilers HMB has been shown to decrease the incidence of sudden death syndrome. Furthermore, HMB increased immune system function and lowered LDL levels in young chicks (Ostaszewski et al., 1998).

Materials and Methods

Present studies were conducted to assess carcass quality of young broilers fed standard diet (starter until 14 d of age; grower between 15 and 42 d of age; finisher between 41 and 49 d of age). All broilers from the experimental group received CaHMB dissolved in drinking water (2 g/10 l; n=150). Broilers from the control group (n=150) were fed the same diet (Table 1) and received water without HMB. At 49 d of age birds were killed and 10 birds from each group were randomly selected for further analysis. In both groups fresh muscles from breast and leg were evaluated for technological parameters like pH, color and water absorption. In fresh muscle tissues protein , dry matter, fat and cholesterol content were also determined. The remainders of muscles were stored for 1 month in -18^0C and then analyzed for the content of

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thiobarbituric acid-reactive substances (TBARS), an indicator of lipid peroxidation. TBARS were assayed by the colorimetric method (Rice-Evans, 1991). In principles, 150 mg of wet tissue was homogenized with PBS (pH=7,4) and then 2.5 ml 0.05M H2SO4 and 2.5 ml 1% thiobarbituric acid were added to homogenate. The mixture was vortexed, incubated for 60 min in boiling water, then extracted with n-butanol. Sample absorbance was determined at 532 nm against blank (n-butanol). TBARS concentration, expressed as malondialdehyde (MDA), was calculated from a standard curve using 1,1,3,3-tetramethoxypropane which gives MDA upon hydrolysis during the assay.

Table 1. Diet composition

Ingredient (% as fed)	Starter	Grower	Finisher
Corn	32	30	30
Wheat + enzyme	22,9	10,9	11,8
Triticale + enzyme	3,0	6,0	6,0
Barley + enzyme	3,0	6,0	6,0
Wheat bran + enzyme	-	6,0	6,0
Soya bean meal (47% CP)	22,4	20,0	13,4
Rape seed meal (33% CP)	2,0	5,0	10,0
Meat meal (55% CP)	5,0	5,0	-
Meat meal (60% CP)	5,0	5,0	10,0
Soya bean oil	2,0	4,0	4,6
Dicalcium phosphate (18% P)	0,4	0,1	0,2
DL-methionine	0,2	-	-
Lider Starter premix	2,1	-	-
Lider Grower premix	-	2,0	-
Lider Finisher premix	-	-	2,0

CP – crude protein; P - phosphorus

Results and discussion

Body weight and selected parameters of body composition are presented in Table 2. Protein and dry matter content in fresh breast and leg muscles were similar in both groups. HMB administration increased fat content in breast muscles (NS). In turn muscle cholesterol content was significantly lower in HMB-treated birds when compared to controls (decrease by 22 and 29% for fresh breast and leg muscles, respectively; $P<0.05$) (Table 3). TBARS content decreased 19% in fresh breast and 52% in fresh leg muscles of chicks treated with CaHMB ($P<0.05$). One month storage

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increased TBARS content in muscles of chicks from control group, whereas in HMB group muscle TBARS content remained the same (Table 4).

Table 2. Body weight, carcass weight and weight of breast and leg muscle of 49-day-old broilers receiving HMB.

Group	Body weight before slaughter (g)	Carcass weight (g)	Weight of breast muscle (g)	Percentage of breast muscle in carcass (%)	Weight of leg muscle (g)	Percentage of leg muscle in carcass (%)
Control	2450	1677,3	431,4	25,7	371,4	22,1
HMB	2381	1643,6	438,8	26,7	333,0	20,3

Table 3. Total cholesterol content (mg/100 g) in selected muscles of 49-day-old broilers receiving HMB.

Group	Breast muscle	Leg muscle
Control	$74,51^{a}$	$86,64^{a}$
HMB	$58,21^{b}$	$61,58^{b}$

^{a,b}Values with different superscript differ significantly ($P<0,05$).

Table 4. TBARS content (mg MDA/kg) in fresh muscles and in muscles stored for 1 month in -18^{0}C.

Group	Fresh muscles		Muscles stored for 1 month	
	Breast	leg	Breast	leg
Control	$0,578^{c}$	$2,118^{d}$	$0,946^{a}$	$2,960^{e}$
HMB	$0,473^{b}$	$1,021^{a}$	$0,550^{b}$	$1,073^{a}$

^{a,b,c,d,e}Values with different superscript differ significantly ($P<0,05$).

Cholesterol is one of many agents generating *in vivo* oxidation of various substrates. In our studies decreased cholesterol content observed in HMB group could indirectly support inhibitory effect of HMB on lipid oxidation expressed as decreased TBARS content in that group.

In conclusion, our studies show that HMB administered in drinking water lowers muscle cholesterol content and inhibits lipid peroxidation in muscles of young broilers. Since this is first report indicating that HMB might be used as a natural antioxidant in poultry industry further *in vitro* studies are needed to confirm that observation.

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MEAT FATTY ACID COMPOSITION IN MALE DUCKLINGS FED DIETS SUPPLEMENTED WITH *Crypthecodinium cohnii.*

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Keywords : PUFA, DHA, duck, alga, *Crypthecodinium cohnii*

Abstract

The objective of this investigation was to study the possibility to increase the docosahexaenoic acid (DHA) content of Muscovy duck meat by supplementing the diet with the microalga *Crypthecodinium cohnii*. Two diets were provided to 58 male ducks, belonging to an Italian rural strain, from the 7th to the 10th week of life: a corn-soybean based diet as the control diet and the same diet supplemented with 0.5% microalga meal. Dietary treatment did not induce differences in growth performances and slaughtering yields. Similarly, chemical composition, colour, pH and oxidative stability of breast muscle were not influenced by the diet. DHA content of breast and liver was significantly increased by the experimental diet.

Résumé

Cette étude visait à augmenter la teneur du muscle en acide docosa-hexaenoïque (DHA), chez le canard de Barbarie, grâce à un régime enrichi avec la microalgue *Crypthecodinium cohnii*. Deux régimes ont été testés sur 58 canards mâles, de souche fermière italienne, entre les âges de 7 et 10 semaines : un régime témoin à base de maïs et de tourteau de soja et le même régime enrichi avec 0.5% de farine de microalgue. Le régime alimentaire expérimental n'a pas induit de modification de la croissance ni du rendement en carcasse. La composition chimique, la couleur, le pH et la stabilité oxydative du filet n'ont pas non plus été influencés par le régime. Le contenu en DHA du filet et du foie a augmenté chez les animaux du groupe expérimental.

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Introduction

In human nutrition the biological effect of n3 long chain polyunsaturated fatty acids (LC-PUFAs) has received a great deal of interest as they play an active role in the prevention and management of several pathologies as coronary heart disease, hypertension, type 2 diabetes, renal disease, ulcerative colitis, chronic obstructive pulmonary disease and Crohn's disease (Simopoulos, 2000). The main molecules of the n3 LC-PUFAs family involved in the beneficial biological effects are eicosapentaenoic acid (EPA, 20:5n3), docosapentaenoic acid (DPA, 22:5 n3) and docosahexaenoic acid (DHA, 22:6n3). The current Western diet is characterised by a poor fish consumption, which represents the main source of these n3 LC-PUFAs, while products of terrestrial animal origin are characterised by higher amount of PUFAs of the n6 series (Simopoulos, 2000). The nutritional value of poultry meat could be enhanced by the increase of the n3 LC-PUFAs content.
Several studies concerning the possibility to enrich the n3 LC-PUFAs in poultry products have indicated the fish oil as the most effective feed for this purpose (Hargis and Van Elswyk, 1993; Leskanich and Noble, 1997). In our previous experiences we showed the possibility to improve LC-PUFAs amount in duck meat by dietary fish oil (Schiavone et al., 2002). The recommendation of the European Union (decision 00/766/EU), concerning the prohibition of using the proteins of animal origin in animal nutrition, and the consumers preferences have stimulated the interest in developing animal diets composed exclusively by vegetable ingredients.
The objective of this investigation was to increase the docosahexaenoic acid (DHA) content of breast muscle of Muscovy duck fed on a diet supplemented with microalga *Crypthecodinium cohnii*, a non-photosynthetic marine dinoflagellate, characterized by high levels of DHA (Jiang et al., 1999).

Materials e Methods

Muscovy ducklings of Italian rural strain, hatched at the Experimental Avian Station of the Department of Animal Production of Pisa, were used for the trial. Ducklings, weighted individually once a week, were separated by sex at 36 days of age. A total of 48 males were distributed in six pens (4 ducks/m^2). All ducks received the same diet: a starter diet (12.1 MJ ME/kg and 240 g CP/kg) between 0 and 5 weeks of age and a growing diet (12.0 MJ ME/kg and 200 g CP/kg) between 6 and 10 weeks of age. During the last three weeks of life 24 birds fed a modified growing diet enriched with 0.5% dried microalga *Crypthecodinium cohnii* (MA diet); the other 24 birds fed the unmodified diet (C diet). The two diets were isocaloric and isonitrogenous. Olive oil

was used as fat source in all the diets. Individual body weights and feed consumption per pen were recorded weekly to calculate the feed conversion ratio (FCR).
Birds were starved for 12 hours before slaughtering and 7 ducks per each dietary treatment were sacrificed. Plucked and eviscerated carcasses were weighted after removal of the head, neck, feet and abdominal fat to obtain ready-to-cook carcasses and refrigerated for 6 hours at 4°C.
Yields from chilled carcass, breast and leg-thigh were evaluated. Breast samples were taken and meat colour and pH measurements were performed. Meat colour (CIELAB system: *L*, a*, b**) was determined on the surface of breast muscle using the Minolta Chroma-Meter CR-300 colour analyser. pH measurements were taken using a Hanna Instruments 8417 pH-meter supplied with a Hamilton Biotrode electrode. Breast samples were vacuum-packaged and frozen (-20°C) until analyses were performed: chemical composition, fatty acid composition and evaluation of susceptibility to lipid oxidation by means of thiobarbituric acid reactive substances (TBARS). AOAC methods (1990) were used for moisture, ash, protein and ether extract determination of breast samples; results are expressed as percentage on fresh matter basis. The fatty acid composition of breast muscle, liver and diets were determined by capillary gas chromatography after lipid extraction (Folch et al., 1957) and esterification (Christie, 1982), using sodium methoxide as catalyst. The fatty acid methyl esters were analysed by a Perkin-Elmer AutoSystem gas-chromatograph, equipped with a flame ionisation detector and a Supelco Omegawax 320 capillary column (30 m x 0.32 mm, 0.25 μm film). TBARS evaluation was performed according to the procedure described by Huang and Miller (1993). Briefly, 3 g of minced breast were homogenized in 57 ml of a 1.15% KCl chilled solution. 30 ml of the homogenate were incubated at 37°C in shaking water bath with 8.34 mg $FeSO_4 \cdot 7H_2O$ (final concentration 1 mM Fe^{+3}) as oxidative agent. TBARS assay was performed at 0, 60, 120 and 180 minutes of incubation. Results are expressed as milligrams of malondialdehyde (MDA) per kilogram of meat.
Data were analysed by Analysis of Variance, using the diet as source of variation (SPSS, 1997).

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Results and discussions

Table 1: growth performances, slaughtering yields and breast meat characteristics (mean ± s.d.) (n = 7)

	C diet	MA diet	diet
Body weight (g)	3314±209	3339±215	NS
F.C.R. (36d-70d)	4.1±0.15	4.4±1.03	NS
Carcass weight (g)	2162.9±66.04	2180.7±40.58	NS
Carcass yield (%) [1]	62.8±1.50	63.1±1.29	NS
Legs-Thighs (g)	459.9±16.85	472.8±16.29	NS
Legs-Thighs (%) [2]	21.3±0.71	21.7±0.97	NS
Breast (g)	431.2±22.70	432.2±50.00	NS
Breast (%) [2]	19.9±0.64	19.8±1.98	NS
Breast characteristics:			
Colour			
L*	45,3±1,58	46,7±1,73	NS
a*	16,0±0,89	15,7±0,82	NS
b*	2,3±0,86	2,7±1,35	NS
pH	5,75±0,10	5,69±0,06	NS
Chemical composition			
moisture (%)	76.77±0.187	76.57±0.368	NS
protein (%)	20.59±0.849	20.17±0.199	NS
ether extract (%)	0.99±0.247	1.03±0.147	NS
ash (%)	1.23±0.076	1.26±0.48	NS

[1] on body weight

[2] on ready-to-cook carcass weight

The fatty acid composition of microalga meal was characterised by the 30.6% of DHA. The inclusion of 0.5% of microalga meal in MA diet brought out DHA content to 0.23% of total fatty acids, while DHA was not detected in the C diet.

The growth performances, slaughtering yields and meat characteristics of breast muscle (colour, pH and chemical composition) were not influenced by dietary treatment (table 1).

The fatty acid composition of breast meat and liver was affected by the diet and the DHA amount was higher in animals fed on the MA diet. The improvement of DHA significantly influenced the total n3 PUFAs amount and the n6/n3 ratio.

Dietary algae have been successfully used in laying hens nutrition to obtain enriched eggs in DHA or eicosapentaenoic acid (EPA) (Herber and Van Elswyk, 1996; Nitsan et al., 1999; Sirri et al., 2001).

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Table 2: fatty acid composition (% of fatty acid methyl esters) of breast and liver (mean ± s.d.) (n = 3) *(** P<0.01; *** P<0.001)*

	breast			liver		
	C diet	MA diet	diet	C diet	MA diet	diet
C14:0	0.4±0.06	0.3±0.08		0.4±0.18	0.3±0.11	
C16:0	24.0±0.24	22.4±1.33		23.7±0.22	22.7±0.07	**
C16:1n7	2.1±0.56	1.4±0.96		1.1±0.34	0.6±0.24	
C18:0	12.3±0.79	13.2±1.69		19.2±0.30	20.6±1.17	
C18:1n9	23.7±1.76	23.3±3.85		19.0±2.53	17.3±3.65	
C18:1n7	2.6±0.34	2.4±0.19		1.6±0.12	1.5±0.11	
C18:2n6	15.1±0.56	16.7±2.69		7.2±0.56	8.2±1.41	
C18:3n6	0.1±0.01	0.1±0.02		0.1±0.04	0.1±0.03	
C18:3n3	0.3±0.06	0.3±004		0.1±0.03	0.2±0.02	
C20:0	0.1±0.03	0.2±0.04		0.1±0.01	0.1±0.00	
C20:3n6	0.8±0.01	0.8±0.23		1.3±0.29	1.2±0.41	
C20:4n6	9.9±0.26	9.8±1.01		17.0±2.01	17.2±1.34	
C20:3n3	0.0±0.03	0.1±0.02		-	-	
C20:5n3	0.1±0.01	0.1±0.03		0.1±0.04	0.1±0.05	
C22:4n6	2.1±0.20	1.8±0.25		1.6±0.20	1.5±0.27	
C22:5n6	1.2±0.26	1.0±0.04		2.4±0.63	1.6±0.04	
C22:5n3	0.5±0.02	0.4±0.09		0.2±0.05	0.2±0.04	
C22:6n3	0.7±0.09	2.1±0.43	**	1.7±0.45	3.7±0.64	**
SFAs	37.0±0.79	36.3±0.45		43.6±0.23	43.9±1.13	
UFAs	60.3±1.71	61.5±0.71		54.0±0.26	54.0±0.69	
SFAs/UFAs	0.6±0.03	0.6±0.01		0.8±0.00	0.8±0.03	
MUFAs	29.1±1.94	27.8±4.64		21.9±2.65	19.6±3.95	
PUFAs	31.2±0.52	33.7±4.38		32.1±2.89	34.5±3.28	
n6	29.5±0.50	30.7±3.91		29.9±2.45	30.3±2.65	
n3	1.7±0.13	3.1±0.51	**	2.2±0.44	4.2±0.66	**
n6/n3	17.8±1.51	10.0±0.66	***	13.8±1.49	7.2±0.62	***

The susceptibility to lipid oxidation (table 3) was not affected by the diet, even if mean values of animal receiving the MA diet were slightly higher. The improvement of DHA amount could be related to this result, as the susceptibility to oxidation of unsaturated fatty acids is related to both the lipid chain length and the number of double bonds (Liu et al., 1997).

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Table 3: TBARS assay on breast muscle (mg of MDA/kg meat) (mean ± s.d.) (n = 7)

	0’	60’	120’	180’
C diet	0,9±0,55	1,9±0,92	1,6±0,65	1,5±0,67
MA diet	1,2±0,60	2,5±1,03	2,2±0,78	2,0±0,76

In conclusion, the inclusion of 0.5% of microalga meal did not negatively influenced the productive traits of the animals. The dietary microalga meal of the present study represents an interesting way to improve DHA content in duck meat using a diet formulated without animal sources. This DHA-enriched meat can have a place in a healthful diet and give an important contribution to a good nutrition, providing three-fold of the DHA content comparing to unenriched duck meat.

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COMMERCIAL CHICKEN MEAT PRODUCTION IN GREECE: THE 'PHYTOAROMA' CHICKEN MEAT

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Keywords: 'Phytoaroma' chicken meat, commercial production

Abstract

The increased consumer's demand for healthy and nutritionally rich chicken meat has led to the introduction of new feeding applications. To that direction natural products such as herbs are currently used. We studied the use of a mixture of such products, hence called 'phytoaroma', to the broilers' ration of a company located in a rural territory of the Northern Greece. Meat produced from chickens fed on 'phytoaroma', represents the 11.44% of the company's total chicken meat production. Although the conventionally produced chicken meat from the company is retailed at the rural markets the 'phytoaroma' chicken meat is sold mainly in the urban markets to portions 7.7% and 92.37%, respectively. Such difference in preference can be ascribed to the different perspectives of the consumer inhabitants in those areas.

Résumé

Les exigences croissantes des consommateurs concernant une viande de poulet saine et substantielle ont contribué à de nouvelles applications au sujet de l'alimentation des volailles. C'est pour cette raison que des produits naturels comme les plantes aromatiques, sont utilisés actuellement. On a étudié l'utilisation d'un mélange de tels produits, nommé 'phytoaroma' dans les rations des poulets destinés à la production de viande dans une entreprise de la Grèce du Nord. La viande produite avec des poulets nourris au 'phytoaroma' représente 11,4% de la production totale de la viande de poulet de l'entreprise. Bien que le poulet produit conventionnellement par l'entreprise se vende dans les marchés ruraux, le poulet 'phytoaroma' alimente principalement les centres urbains avec un taux de 7.7% et de 92.3%, respectivement. Cette divergence des choix est due aux différentes préférences des consommateurs qui habitent dans ces régions.

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Introduction

The poultry sector of the global meat supply has experienced a dynamic growth in production and consumption in most countries over the last years. Especially, in developing countries, poultry meat production has exceeded that of the developed ones with chicken meat representing the imposing majority of the total poultry meat output. In most of these markets, the consumers prefer chicken meat due to its lower price, perceived safety and health advantages compared to other meat sources.

Introduction of new products that meet the market chain requirements and consumer needs have contributed significantly to the increases in global chicken meat consumption. In Greece and in most European countries, paralleling economic prosperity, chicken meat has an important role in human's diet.

Consumers' considerations in health and nutritional aspects of food are the most important factors influencing their choices (Tserveni- Gousi et al., 2002). Under this spectrum, functional foods, products in addition to their nutritive value having health promoting benefits (Blades, 2000), have been introduced.

The 'phytoaroma' chicken meat is produced under certain nutritional principles (plant feeding and addition of a mixture of herbs; GR1003646, 2001) and is available to the Greek market under indicative label. Combining the beneficial effects of the natural herbs and an improved flavour, this type of chicken meat was well accepted by the consumers (Yannakopoulos et al., 2001; Tserveni- Gousi et al., 2002).

The production and market analysis in a new type of chicken meat under commercial conditions was the objective of the present, preliminary study.

Materials and Methods

'Phytoaroma' chicken meat is exclusively produced from a small/medium size enterprise, vertical integrated, which is located in a rural territory of Northern Greece. In order to evaluate the consumer trend in relation to the new type of chicken meat, sales data for a seven- month period (from June to December of the year 2002) were collected. This period is important for the Greek market and is characterized by changes in chicken meat consumption due to seasonal changes mainly. These changes reflect the seasonality of physical conditions and the fluctuation of the chicken meat distribution.

It should be mentioned here that the company supplies with both conventional and 'phytoaroma' chicken meat supermarkets, butcheries and retailers in several major markets of Greece.

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Urban markets in the present study were considered the markets of Athens and Thessaloniki (the two major cities in Greece), while markets in other areas were all considered as rural ones.
Descriptive statistical methods were used for the analysis of the data.

Results and discussion

Table 1 reports the sales data about the conventional and the 'phytoaroma' chicken meat. According to these data, 'phytoaroma' chicken meat represents the 11.44% of the company's total sales in chicken meat.

Table 1. Sales data on conventional and 'phytoaroma' chicken meat during the selected period

Period	Chicken meat sales (kg)		
	Conventional	'Phytoaroma'	% Of conventional
June	94553.8	10816.0	10.26
July	101051.7	9866.0	8.89
August	109555.0	7684.0	6.55
September	102103.5	14305.0	12.29
October	94458.8	13656.0	12.63
November	87332.0	14675.0	14.39
December	91489.7	15536.0	14.52
MEAN	**94530.4**	**10817.4**	**11.44**

An increase of the sales is noticed for the conventional chicken meat (Fig. 1), but the opposite trend is observed to the sales of 'phytoaroma' chicken meat during the summer months and especially in August. The reason of the reduction in the sales of 'phytoaroma' chicken meat can be ascribed to the move of the inhabitants from urban areas in rural ones where the major tourist centers are located. It is important to notice that in the following period (September to December) the sales of 'phytoaroma' chicken meat increase and remain stable at higher levels.

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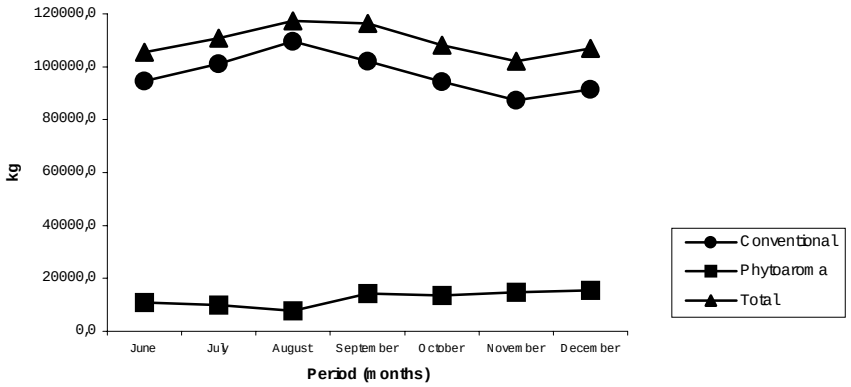

Fig. 1. Sales of the conventional and the 'phytoaroma' chicken meat

The sales of the conventional chicken meat in the islands of Lemnos and Syros, islands preferred by the Greek tourists mainly, are given in Fig. 2. An increase of the conventional chicken meat sales is noticed also in August supporting thus the previous findings.

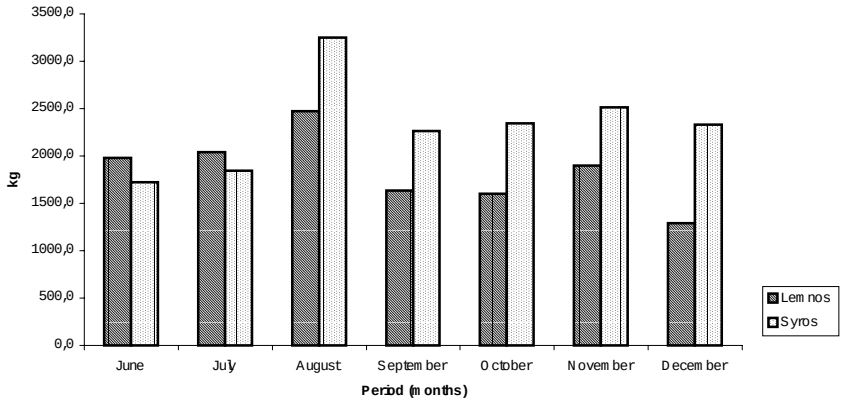

Fig. 2. Sales of the conventional chicken meat in two Greek islands

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Although the conventionally produced chicken meat from the company is retailed at the rural markets (data not presented here) the 'phytoaroma' chicken meat is sold mainly in the urban markets. According to the data collected, 92.3% of the total sales are taking place in the urban markets of Athens and Thessaloniki and only 7.7% in the rural area markets.

The sales of 'phytoaroma' chicken meat in urban and rural areas are given in Fig. 3.

The results reveal that the major volume of the 'phytoaroma' chicken meat is retailed in the market of Athens (63.49%) compared to the markets of Thessaloniki (28.81%) and of the rural areas (7.7%). Furthermore, the sales of 'phytoaroma' chicken meat in the market of Thessaloniki are increasing from October to December implying thus the consumers' acceptance towards this product. As expected, the lowest sales rates in all areas were observed in July and August.

The wholesale price of 'phytoaroma' chicken meat, set by the company, is about 16% higher from the price of the conventional chicken meat due to the higher cost of the natural additives. This price remained constant during the period studied and it seems that did not affect the consumers' behaviour towards 'phytoaroma' chicken meat.

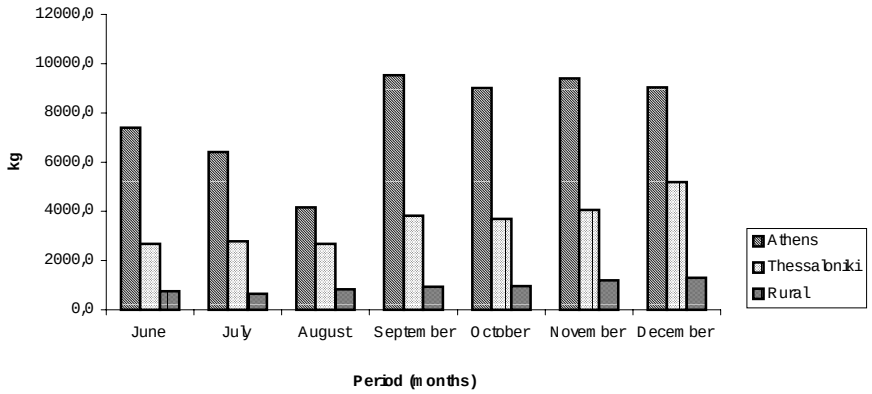

Fig. 3. Sales of the 'phytoaroma' chicken meat in urban (Athens, Thessaloniki) and rural areas

There is an ongoing research over the past few years on meat consumption and especially on consumer behaviour and attitude towards meat consumption (Verbeke and Viaene, 1999). In most of the studies the need for more and better consumer information on meat quality is underlined. Consumers, regardless their regional origin and preferences on meat consumption concern about matters related to food safety and health and expect the responsiveness by the meat production sector.

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In conclusion, the results from the present preliminary study suggest a further approach to the needs of the final consumers and especially to their behaviour towards new products presented to them.
Enterprises interested in improving a quality scheme should focus on these needs.
Chicken meat producers can invest in the production of new products following acceptable production methods and through a cautious trade policy can also earn the trust of the consumers.

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CONTENTS

EFFECT OF GENOTYPE AND FORCE-FEEDING ON THE INTRAMUSCULAR FAT DEPOSITION IN DUCK

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Keywords : duck, meat, muscle, lipid, fatty acids, quality

Abstract

We conducted a study to evaluate the effects of genotype (Muscovy, Pekin and their crossbred, mule and hinny) and force-feeding on intramuscular fat (IMF) deposition in duck. Our findings showed that force-feeding and genotype exerted significant effects on IMF quantity and quality (lipid classes and fatty acids). The main differences were observed between Muscovy and Pekin ducks, while crossbred ducks were intermediate for all parameters, except for body and liver weights.

Résumé

Une étude a été réalisée afin d'évaluer l'effet du génotype (Barbarie, Pékin et leurs croisements, mulard et hinny) et du gavage sur le dépôt de lipides intramusculaires (LIM) chez le canard. Nos résultats montrent que le gavage et le génotype ont des effets significatifs sur la quantité et la qualité (classes de lipides et profil en acides gras) des LIM. Les principales différences sont observées entre les canards Pékins et Barbarie, alors que les croisements sont intermédiaires pour tous les paramètres à l'exception du poids vif et du poids de foie.

Introduction

Two main species of ducks are used in France to produce meat and fatty liver: Muscovy and mule, respectively. Mule duck is the crossbred between the Muscovy male and the Pekin female and gains from heterosis effect for fatty liver production

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(higher weight and lower lipid release after heating). Guy et al. (1999) and Hermier et al. (2000) demonstrated that susceptibility to store lipids in the liver or in peripheral tissues such as adipose tissues and muscles depended on duck genotype. Pekin ducks showed a much marked extrahepatic fattening and Muscovy ducks exhibited the reverse situation. The intramuscular fat (IMF) deposition is involved in determining meat quality, particularly the nutritional and sensorial characteristics and the conservation ability. The aim of our study was therefore to analyse the effect of genotype (Muscovy, Pekin and their crossbred, mule and hinny) and force-feeding on the quantity and quality (lipid classes, fatty acids) of IMF deposition in duck.

Materials and methods

Four different genotypes of male ducks, Pekin, Muscovy and their crossbred, mule and hinny (50 ducks per genotype issued from the same sires and dams, Grimaud Company, Roussay, France) were reared under natural conditions of light and temperature at the Experimental Station for Waterfowl Breeding (INRA Artiguères, France). From hatching to 6 weeks of age, they were fed ad libitum. From 6 to 12 weeks of age, birds were fed on a restricted diet at levels appropriate to each genotype (200-250 g per duck at the beginning, increasing to 360-380 g at the end of the period). At 12 weeks of age, 30 ducks per genotype were overfed at the maximum of their ingestion potential for 14 days with corn grains and a diet compsed mainly of corn and given as mash (Gavimaïs, table 1). During the overfeeding period, 12 ducks per genotype were kept ad libitum with growing diet (controls). The main characteristics of diets (starting, growing and overfeeding) are shown in table 1.
Growth rate and feed conversion ratio were estimated by the individually weighing of animals and measuring food consumption at different ages. Eight ducks per treatment were sacrificed at 98 days of age. Iliotibialis superficialis (IT, thigh muscle) and Pectoralis major (PM, breast muscle) were excised, weighed and samples were frozen and stored at -20°C. Carcass fatness was estimated by weighing abdominal fat. Force feeding performance was estimated by weighing liver. Lipid content in muscles and diets was measured (Folch et al., 1957). Classes of lipids were determined using Iatroscan (Iatron, Tokyo, Japan) thin layer chromatography with a flame ionisation detector system (TLD-FID) according to Mares et al. (1983). The fatty acid composition was detemined after transmethylation of lipids (Morrison and Smith, 1964), by gas chromatography (Perkin Elmer autosystem, St Quentin en Yvelines, France). Injection and detector temperatures were 250°C, the carrier gas was nitrogen with a head column pressure of 16.5 psi. Methyl esters were identified and quantified by comparison with standards (Sigma, St Quentin Fallavier, France). Data were compared with variance analysis followed by a Newman-Keuls test.

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Results and discussion

Only the interaction between genotype and force-feeding are presented in the tables. The individual effect of each factor will be discussed in the following text.

1. Feed characteristics and feed consumption

Force feeding diet had lower protein content and higher contents of lipid (3.38 vs 2.84 %) and poly-unsaturated fatty acids (PUFA) than growing diet (table 1). Muscovy ducks displayed the lowest feed conversion ratio and consumption of corn or growing diet during rearing and overfeeding periods, respectively (table 2).

2. Growth performance and carcass composition

Because of a late development, Muscovy ducks exhibited the lowest body weight at 4 weeks of age, but the highest at 12 and 14 weeks of age (tables 3 and 4). By comparison with control ducks, force feeding induced a significant increase in body weight (+ 30 %), and weights of liver (7-fold) and abdominal fat (2.2-fold, table 4). Force feeding had no effect on muscle growth. By comparison with other genotypes, Pekin ducks had the lowest liver weight and the highest weight of abdominal fat. Muscovy ducks had the highest weight of muscles (table 4). These observations confirmed previous results of Hermier et al. (2000). Pekin ducks rather accumulated lipids in peripheral tissues while Muscovy ducks exhibited liver fattening. Higher concentrations of plasma insulin measured in Pekin ducks after 10 days of overfeeding could be responsible for the maintenance of the liprotein lipase activity which favours extra hepatic fattening rather than liver steatosis (Davail et al., 2003).

3. IMF deposition in breast (PM) and thigh (IT) muscles

By comparison with control ducks, force feeding induced a significant increase in lipid (1.6 fold in PM and 1.3 fold in IT) and triglyceride (2.0 fold in PM and 1.3 fold in IT) contents of muscles (tables 5 and 6). This effect was more important in breast than in thigh muscle, likely because the growth of this later was achieved before 12 weeks of age. This hypothesis was confirmed by the level of correlations between the percentages of lipid and triglycerides which were higher in PM than in IT: 0.86 vs 0.78. By comparison with other genotypes, Pekin ducks had the highest lipid and triglyceride ($p<0.05$) contents in muscles and Muscovy ducks the lowest. Muscovy ducks exhibited the lowest phospholipid content in both muscles and Pekin ducks, the highest in IT (NS). Force feeding induced an increase in mono-unsaturated fatty acids (MUFA), particularly oleic acid (C18:1) at the expense of PUFA, particularly arachidonic acid (C20:4) suggesting an increase in oxidative metabolism of muscles. This result is consistent with the defect in the incorporation of linoleic acid (C18:2) and linoleic and linolenic-derived PUFA, observed despite the high proportion of these essential fatty acids in the force feeding diet based on corn (Cazeils et al., 1999). These authors concluded that in overfed waterfowl, *de novo* hepatic lipogenesis prevailed over dietary lipid intake to modulate lipid composition of tissues. Gabarrou

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et al. (1996) also suggested lower activities in Δ5 and Δ6 desaturases. By comparison with other genotypes, Pekin duck had the lowest percentages of SFA and PUFA, and the highest percentage of MUFA in muscles. Crossbreed ducks were intermediate for all these parameters.
Future studies will be conducted to better understand the differences observed between genotypes in their ability to store lipids in muscles, to describe the location of IMF deposition (adipocytes and/or muscular fibre) and to determine the relationships between quantity of lipids in muscles and the sensorial quality of meat, particularly flavour, tenderness, juiciness and colour.

Acknowledgements

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Table 1: Main characteristics of feed for rearing and force-feeding periods. Preparation for force-feeding contained corn (25 %), corn meal (= gavimaïs, 35%) and water (40%)

Composition	**Starting (0-4 weeks)**	**Growing (4-12 weeks)**	**« Gavimaïs »**	**Corn**
ME kcal/kg *	2830	2850	3387	3250
CP (%)	18.21	15.98	8.03	8.63
Lipids (%)	3.34	2.84	3.86	2.71
SFA (%)	17.17	16.10	14.49	14.57
MUFA (%)	24.98	28.36	28.33	26.19
PUFA (%)	57.85	55.54	57.17	59.22

*: calculated value for metabolisable energy (ME) – CP = Crude Protein – SFA, MUFA, PUFA = Saturated, Mono-Unsaturated and Poly-Unsatured Fatty Acids

Table 2: Effect of duck genotype on feed conversion ratio (FCR) during rearing period (0 to 84 days), total feed consumption of equivalent dry maize (g) during force feeding period (85 to 98 days) and total feed consumption (g) of control ducks during this same period

Genotypes	**Muscovy**	**Hinny**	**Mule**	**Pekin**
Rearing period (FCR)	2.76	3.23	3.29	3.63
Force feeding period	8219	10341	10552	9674
Feed consumption of control ducks	3112	4027	4295	3923

Table 3: Effect of genotype on growth performance during the rearing period (body weight, g - mean $\pm$ sd - n = 50)

Genotypes	**Muscovy**	**Hinny**	**Mule**	**Pekin**
4 weeks	1257 $\pm$ 92 d	1593 $\pm$ 85 c	1684 $\pm$ 115 b	1741 $\pm$ 124 a
6 weeks	2715 $\pm$ 148 c	2626 $\pm$ 148 d	2846 $\pm$ 209 b	2985 $\pm$ 183 a
12 weeks	4919 $\pm$ 323 a	4588 $\pm$ 395 b	4622 $\pm$ 314 b	4477 $\pm$ 294 b

a - d: significant difference between genotypes for one parameter with $P < 0.05$

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Table 4: Effect of genotype and force feeding on body weight, weight of liver, abdominal fat, Pectoralis major (PM) and Iliotibialis superficialis (IT) muscles of ducks (FF = Force Fed, C = Control, mean ± sd, n = 8)

Genotypes		**Muscovy**	**Hinny**	**Mule**	**Pekin**
Body weight (g)	FF	6393 ± 441 a	6315 ± 402 a	6473 ± 351 a	5999 ± 353 a
	C	5418 ± 245 b	4854 ± 497 c	4455 ± 392 c	4623 ± 426 c
Liver weight (g)	FF	467 ± 90 a	495 ± 85 a	494 ± 97 a	318 ± 75 b
	C	77 ± 7 c	61 ± 6 c	61 ± 13 c	56 ± 11 c
Adominal fat (g)	FF	220 ± 23	256 ± 27	224 ± 42	265 ± 22
	C	109 ± 20	107 ± 35	73 ± 35	155 ± 29
PM weight (g)	FF	408 ± 49	290 ± 25	309 ± 28	208 ± 33
	C	398 ± 39	297 ± 29	278 ± 30	216 ± 18
IT weight (g)	FF	30.1 ± 3.3	23.0 ± 2.5	23.1 ± 3.1	19.0 ± 1.6
	C	32.8 ± 4.4	22.9 ± 3.6	22.3 ± 2.5	19.5 ± 2.8

a - c: significant difference between groups for one parameter with $P < 0.05$

Table 5: Effect of genotype and force feeding on lipid content, lipid classes and fatty acid composition in Pectoralis major muscle of ducks (FF = Force Fed, C = Control, mean ± sd, n = 8)

Genotypes		**Muscovy**	**Hinny**	**Mule**	**Pekin**
Total lipid (%)	FF	3.65 ± 0.51	5.92 ± 0.98	5.24 ± 0.96	7.57 ± 0.85
	C	2.26 ± 0.36	3.87 ± 1.44	3.13 ± 0.54	4.59 ± 0.68
Triglycerides	FF	2.37 ± 0.47 d	4.35 ± 0.76 b	3.66 ± 0.91 c	5.95 ± 0.86 a
(g /100 g)	C	1.02 ± 0.24 e	2.19 ± 0.87 d	1.79 ± 0.32 d	3.09 ± 0.58 c
Cholesterol	FF	0.13 ± 0.05	0.14 ± 0.05	0.16 ± 0.04	0.15 ± 0.02
(g /100 g)	C	0.12 ± 0.04	0.16 ± 0.08	0.13 ± 0.03	0.17 ± 0.03
Phospholipids (g)	FF	1.15 ± 0.17	1.43 ± 0.26	1.42 ± 0.11	1.46 ± 0.13
(g /100 g)	C	1.13 ± 0.10	1.53 ± 0.51	1.21 ± 0.24	1.33 ± 0.15
SFA (%)	FF	38.0 ± 1.2	36.4 ± 1.8	37.9 ± 1.2	33.4 ± 1.3
	C	39.2 ± 1.6	35.4 ± 3.3	36.9 ± 1.8	32.4 ± 1.2
MUFA (%)	FF	46.4 ± 1.6 c	48.5 ± 2.6 bc	45.7 ± 0.9 c	51.6 ± 1.8 a
	C	37.2 ± 2.2 e	42.9 ± 3.4 d	42.2 ± 3.2 d	49.3 ± 2.3 ab
PUFA (%)	FF	15.7 ± 1.4 d	15.1 ± 1.0 d	16.4 ± 1.3 d	15.0 ± 1.0 d
	C	23.6 ± 1.9 a	21.8 ± 1.3 b	20.9 ± 3.3 b	18.3 ± 1.8 c

a-e: significant difference between for one parameter with $P < 0.05$
SFA, MUFA, PUFA = Saturated, Mono-Unsaturated and Poly-unsaturated Fatty Acids

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Table 6: Effect of genotype and force feeding on lipid content, lipid classes and fatty acid composition in Iliotibialis superficialis muscle of ducks (FF = Force Fed, C = Control, mean ± sd, n = 8)

Genotypes		**Muscovy**	**Hinny**	**Mule**	**Pekin**
Total lipid (%)	FF	2.52 ± 0.61	3.99 ± 0.97	3.74 ± 0.61	5.73 ± 0.76
	C	2.16 ± 0.29	3.16 ± 0.61	2.79 ± 0.39	4.55 ± 0.54
Triglycerides	FF	1.54 ± 0.48	2.75 ± 0.82	2.55 ± 0.56	4.20 ± 0.68
(g /100 g)	C	1.30 ± 0.32	2.12 ± 0.56	1.88 ± 0.30	3.39 ± 0.51
Cholesterol	FF	0.11 ± 0.02	0.14 ± 0.03	0.14 ± 0.01	0.17 ± 0.04
(g /100 g)	C	0.12 ± 0.02	0.13 ± 0.03	0.11 ± 0.01	0.15 ± 0.04
Phospholipids (g)	FF	0.87 ± 0.19	1.10 ± 0.16	1.05 ± 0.12	1.35 ± 0.18
(g /100 g)	C	0.73 ± 0.08	0.90 ± 0.07	0.80 ± 0.11	1.00 ± 0.07
SFA (%)	FF	36.19 ± 1.19	35.61 ± 1.39	34.46 ± 1.16	31.43 ± 1.53
	C	36.44 ± 2.85	33.49 ± 1.82	32.82 ± 0.46	28.77 ± 1.28
MUFA (%)	FF	47.45 ± 1.51	49.66 ± 1.63	50.31 ± 0.94	55.00 ± 1.49
	C	45.46 ± 2.17	47.71 ± 1.22	48.43 ± 2.22	54.73 ± 1.96
PUFA (%)	FF	16.36 ± 1.50	14.72 ± 0.84	15.23 ± 1.36	13.57 ± 0.53
	C	18.11 ± 3.71	18.80 ± 0.96	18.75 ± 1.92	16.50 ± 1.33

SFA, MUFA, PUFA = Saturated, Mono-Unsaturated and Poly-unsaturated Fatty Acids

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SKIN COMPOSITION AND LIPID CHARACTERISTICS OF BROILERS SAMPLED AT RETAIL

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Keywords : skin, composition, broiler, fat characteristics

Abstract

Pooled skins of whole broilers collected twice a month at retail for 12 consecutive months (total n=26) were analyzed for composition including hydroxyproline, TBA value and fat melting point, viscosity and fatty acid content. Compositional ranges for moisture, fat, protein and ash were 42.9-57.6%, 31.1-51.1%, 7.1-15.6%, and 0.30-0.66%, respectively. TBA values averaged 1.25 and extracted fat had averages for melting point and relative viscosity of 29.8 C and 17.1 centipoise (at 40 C), respectively. Fatty acid analysis yielded 32.9% saturated (14:0, 16:0, and 18:0) and 67.1% unsaturated (16:1, 18:1, and 18:2). Composition variation was due to shifts in moisture content, likely related to water retention on chilling. Fat composition and physical fat characteristics remained in a narrow range of values.

Résumé

Des échantillons de peaux de poulets entiers collectés deux fois par mois pendant 12 mois consécutifs (au total n=26) chez des détaillants, ont été analysés pour les critères suivants : hydroxyproline, TBA et point de fusion de la graisse, viscosité et profil en acides gras. Les plages obtenues pour les teneurs en humidité, lipides, protéines et cendres étaient de 42.9-57.6%, 31.1-51.1%, 7.1-15.6%, et 0.30-0.66%, respectivement. La valeur TBA était en moyenne de 1.25 et les lipides extraits ont donné des valeurs pour le point de fusion et la viscosité relative de 29.8°C et 17.1 centipoise (à 40° C), respectivement. Le profil en acides gras était le suivant : 32.9% d'acides gras saturés (14:0, 16:0, et 18:0) et 67.1% d'insaturés (16:1, 18:1, et 18:2). Les variations de composition sont dues à des fluctuations de la teneur en eau, probablement à mettre en relation avec la rétention en eau durant le ressuage. Sinon, la composition et les caractéristiques physiques des lipides ne varient pas dans des proportions importantes.

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Introduction

Market increases in further processed poultry have resulted in excess supplies of chicken skin. Skin collected under regulatory inspection has been used as an ingredient in processed meat formulations and for production of edible fat after rendering (Piette et al., 2001). Composition studies of poultry skin, including those of lipid fatty acids, show variation due to species, age, body location, diet and method of carcass processing. On a wet basis, skin generally contains 35-45% fat (Bonifer et al., 1996; van Heerden et al., 2002) and the fluctuations in moisture content of skin of water chilled carcasses (Sams, 2001) would be expected to influence the content of other constituents such as protein and minerals (ash). The objective of this study was to determine composition and some characteristics of the fat component of skin from marketplace broilers sampled in a 12-month period.

Materials and Methods

Two whole fresh broilers were obtained at 14-day intervals (n = 26) over 12 months from a local market receiving product from a regional processing plant. All skin of was removed, combined, ground and held at 2 C for 12-18 h before sampling and analysis for 2-thiobarbituric acid (TBA) values. Remaining ground skin was stored in sealed containers at –20 C until analyzed for other constituents.

The following analyses were conducted: •*TBA values*. Duplicate 10 g samples of skin were analyzed using the distillation procedure of Tarladgis et al. (1960). •*Moisture*. Stored frozen skin from each collection period was thawed at 2 C and analyzed in duplicate. Samples were dried in Al pans placed in a Virtis 25SL freeze-dryer. •*Fat*. Dry residues from freeze-drying for moisture were extracted using a Soxhlet extractor following Method 39.1.08 of the AOAC (1995). Percent fat was expressed on a wet weight basis of original samples. •*Protein*. Duplicate samples were analyzed for protein (N x 6.25) following AOAC (1995) procedure 39.1.15 Alternative II. •*Ash*. Residues of the duplicates from fat analysis were ashed 500 C following AOAC (1995) Method 39.1.09. Percent ash was calculated on a wet basis. •*Collagen*. Collagen was based on hydroxyproline content of the moisture- and fat-free residues from fat extraction after hydrolysis with 6N HCl under vacuum at 100 C for 22 hr (Moore, 1963). Hydrolysates were analyzed by ion-exchange chromatography using a Phoenix K-8000 VG Amino Acid Analyzer (Phoenix Precision Instruments) (Moore et al., 1958). Hydroxyproline content (mg/g) was calculated on a wet basis and converted to collagen using a factor of 7.57 (Bonifer and Froning, 1996). •*Melting Point*. Skin fat from fat analysis was used to determine melting point. After filling a capillary tube (1

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mm diameter) with fat at 38-40 C, one end was sealed and the sample cooled to 0 C. The capillary was placed in an Electrothermal Melting Point Apparatus (Electrothermal Engineering) and viewed through melting as the temperature increased at approximately 2.5 C/min. Temperature increase was adjusted to approximately 1.5 C/min near the melting point. Melting point was defined as the temperature at which fat became completely transparent from its initial opaque appearance. •*Viscosity*. Extracted skin fat was analyzed for viscosity (centipoise) at 40 C using flow times in two No. 400 Cannon-Fenske viscosimeters previously standardized for constants with distilled water and absolute ethanol at 40 C (Daniels et al., 1962). Fat density at 40 C was 0.897 g/mL determined using weight of samples in 50 mL volumetric flasks. Temperature for density and viscosimeter measures was maintained using HAAKE Model FS water circulator (HAAKE, Inc.) attached to a cylindrical clear glass vessel for immersion of the volumetric flasks and viscosimeters, respectively. •*Fatty Acid Analysis*. Extracted fat was analyzed for fatty acid content using the methylation, gas chromatography and identification methods of Jen et al. (1971). •*Data Analysis*. Means with standard deviations were calculated for all analyses.

Results and discussion

Mean percentages from proximate composition for moisture, fat, protein and ash of broiler skin are shown in Figure 1 and ranges for these constituents (n = 26) were 42.9-57.6%, 31.1-51.1%, 7.1-15.6%, and 0.30-0.66%, respectively. These ranges are slightly larger than found when examining analysis results of van Heerden et al. (2002), Piette et al. (2001) and Bonifer et al. (1996).

An average TBA (Table 1) of 1.25 mg/kg skin was obtained approximating the value reported for broiler thigh meat after 4 days at 4 C (King and Zeidler, 1996). Although post-slaughter history of broilers selected for the study is unknown, they were marketed in loose-fitting, aerobic type packaging under refrigeration in retail display cases. The data does not indicate major oxidative development of the skin lipid. Skin collagen content (Table 1) was in agreement with that of Bonifer et al. (1996) of 40.1 mg/g. Other reported values of 29.5 and 30.5 mg/g from Satterlee et al. (1971) and Swatland and Barbut (1991), respectively, likely reflect differences in sources of skin as well as differences in moisture of initial samples.

Although the reported melting point is 29.8 C (Table 2), extracted skin fat showed 3 distinct visual zones during heating. There was initial clearing at approximately 16.5 C, followed by half transparency at 25 C and complete transparency, defined as the melting point, at 29-30 C. Our melting point is within the 26.7-43.4 C range given for poultry abdominal fat by Keeton (2001) and slightly less than that of beef tallow (40-48 C) reported by Triebold and Aurand (1963) and of lard (33-46 C) by Aurand et al.

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(1987). Viscosity (Table 2) of the skin fat was slightly less than the measured viscosity of commercially refined corn oil which was 19.5 cp at 40 C. No data for viscosity was found in the literature for comparison to these results.

Fatty acid analysis yielded 32.9% as saturated (14:0, 16:0, and 18:0) and 67.1% as unsaturated (16:1, 18:1, and 18:2). A similar comparison for tallow and lard show approximate percent ratios of 70:30 and 53:47, respectively. The higher level of unsaturates is reflected by the lower melting point of skin fat as previously stated. Three fatty acids, 16:0, 18:1 and 18:2, represented 82% of the fatty acids of skin (Figure 2) in direct agreement with that of van Heerden et al. (2002). These three represented 86.2% of the total fatty acids for chicken skin in the study by Kallio et al. (2001) who also reported on regioisomerism of the triacylglycerol (TAG) structures. Within a wide variety of TAG structures found in the skin lipids, analysis of the most frequently occurring molecular weight species showed that 16:0 occurred predominantly in the *sn*-1/3 positions and 18:1 and 18:2 in the *sn*-2 position.

Overall composition of broiler skin and physical characteristics of the skin fat agreed with prior data from the literature. Viscosity of the skin fat, not previously reported, was slightly lower than that of corn oil and this will be of value to the foodservice industry where rendered fat may be used as an ingredient in cooking.

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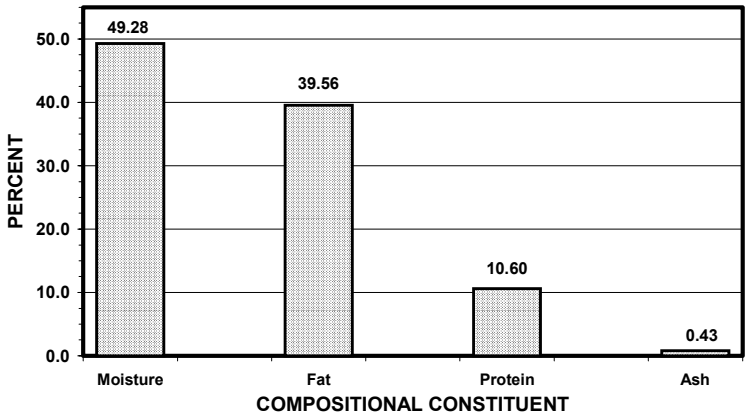

FIGURE 1. Proximate composition of the skin from fresh broilers at retail sampled at 14-day intervals over a 12-month period.

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TABLE 1. Collagen and 2-thiobarbituric acid (TBA) of skin from fresh broilers at retail sampled at 14-day intervals over a 12-month period.

Constituent	Concentration
TBA Value	1.25 ± 0.52 mg/kg
Collagen	42.30 ± 7.13 mg/g

TABLE 2. Melting point and viscosity of skin fat extracted from fresh broilers at retail sampled at 14-day intervals over a 12-month period.

Physical Property	Measure
Melting Point	29.8 ± 1.4 C
Viscosity (40 C)	17.1 ± 3.3 cp

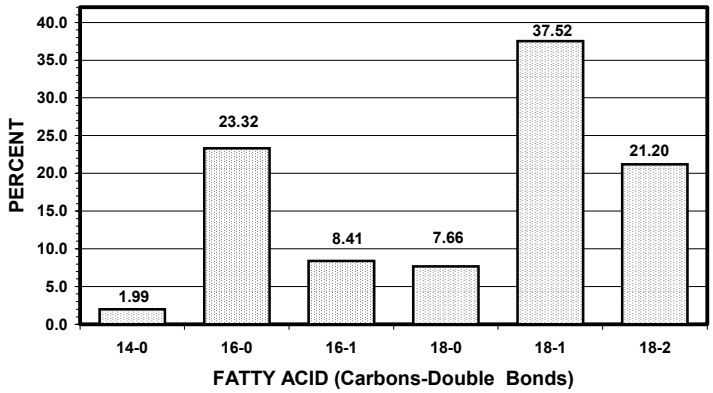

FIGURE 2. Fatty acids of skin fat extracted from fresh broilers at retail sampled at 14-day intervals over a 12-month period.

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EFFECT OF FATTY ACID CONTENT OF GROWER-FINISHER FEED ON FATTY ACID CONTENT OF BREAST MEAT.

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Keywords: n-3 fatty acids, docosahexaenoic, meat enrichment

Abstract

The relation between feed-fatty acids (FA) content and breast meat FA content, as well as performance parameters, were studied in broiler chickens. A total of 240 21d-old birds in cages (5 animals per cage) were fed with diets varying in the content of soybean oil (0 to 2.2%), linseed oil (LO) (0 to 2%) and Capsomega (0 to 0.4%; an algae with 20% docosahexaenoic FA –DHA). Neither performance parameters nor total fat content of breast meat were significantly affected by dietary treatment. Linolenic and n-3 FA content of breast meat were higher with increasing levels in the diet (response could be approximated by linear regression); the response of DHA varied depending on the LO content of the diet: it was not affected when diet contained 1% LO, and it significantly increased when diet contained 2% LO (response could also be approximated by linear regression). The ratio n-6 to n-3 FA in breast meat was 7.8 in soybean oil diet fed animals and 2.0 in 2% LO + 0.4% Capsomega fed animals; for these treatments total n-3 FA was 123 and 412 mg/200 g ration of breast meat, and DHA 23 and 96 mg/200 g ration of breast meat respectively.

Résumé

Les relations entre la teneur en acides gras (AG) de l'aliment et celle des filets de poulets de chair, ainsi que les critères de performance ont été étudiées. Deux-cent quarante poulets âgés de 21 jours logés en cages (5 animaux par cage) ont été nourris avec des régimes différents par la teneur en huile de soja (0 à 2.2%), huile de lin (LO) (0 à 2%) et Capsomega (0 à 0.4%; une algue avec 20% d'acide docosahexaenoïque - DHA). Le régime alimentaire n'a pas eu d'effet significatif sur les critères de performance ni sur la teneur en lipides totaux des filets. Les teneurs des filets en acide

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linolénique et en AG n-3 croissaient avec l'augmentation des niveaux dans les régimes (réponse de type linéaire); la réponse du DHA variait selon la teneur du régime en LO: elle n'était pas significativement affecté quand le régime contenait 1% LO, par contre elle augmentait significativement quand le régime contenait 2% LO (réponse de type linéaire également). Le rapport des AG n-6 sur n-3 dans le filet était de 7.8 avec l'aliment contenant l'huile de soja et 2.0 avec l'aliment contenant 2% LO + 0.4% Capsomega; pour ces traitements la teneur en AG n-3 était de 123 et 412 mg/200 g de filet de poulet et celle en DHA, de 23 et 96 mg/200 g de filet de poulet respectivement

Introduction

Scientific evidences on the beneficial effects of n-3 FA have been accumulated for the last half century: lowering of plasma triglycerides, increased aggregation time for platelets, decreased viscosity of blood, decreased blood pressure, reduction in atherosclerosis, reduction of inflammation, reduction in tumors (Mishra et al., 1993). Chicken meat has been shown to modify its FA profile according to FA in the diet (Hulan et al., 1984; Scaife et al., 1994; López-Ferrer et al., 1999a, b). In order to increase the amount of n-3 long-chain FA (C≥20), linolenic FA sources are less effective than DHA or eicosapentaenoic FA sources, although some strategies may confer low palatability to chicken meat due to strange flavours and taste (Hargis and Van Elswyk, 1993; López-Ferrer et al., 2001a).
The combination of a linoleic FA source (linseed oil) and a DHA source (Capsomega) at different levels in grower-finisher broiler diets was used in the present experiment to assess the enrichment in n-3 FA (specially long-chain FA) of chicken breast meat.

Material and Methods

A total of 240 1d-old male Ross broiler chickens, were fed a corn-based commercial starter diet till 21d. From 1 to 6d old, birds were located in 2 floor pens, and at 6d birds were randomly located in cages (5 animals per cage). At 21d, treatments were randomly assigned to cages taken into account spacial distribution (level and orientation of cages). Treatments consisted in eight diets varying in the content of soybean oil (0 to 2.2%), linseed oil (LO) (0 to 2%) and Capsomega (0 to 0.4%; an algae with 20% docosahexaenoic FA –DHA). Control diet (with 2.2% soybean oil + 0% LO + 0% Capsomega) was formulated to meet or exceed NRC-1994 requirements; LO entered in the formula replacing soybean oil at 1:1; Capsomega

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entered in the formula replacing soybean oil and soybean meal at 2:1:1. Treatments are detailed in Table 1.
Body Weight (BW) and Feed Consumption (FC) was recorded at 21d and 42d. At 42d, 4 animals per treatment (from different cages) were individually identified and sacrificed in a commercial slaughterhouse. Carcasses were stored 24h at 4ºC, when they were hand-cut and commercial breasts cuts recovered (Hudspeth et al., 1973; Orr et al., 1984). Tissue samples for FA analysis were weighted, freeze-dried (FTS Systems model Alpha 1/63, Co Christ, D-37520 Osterode, Germany), and dry matter calculated. Diet and breast meat total lipids were extracted following Folch et al. (1957) method and were methylated with 20% boron trifluoride methanol complex in methanolic solution (Morrison and Smith, 1964). The lipid composition was determined by gas chromatography in a Shimadzu GC-14A chromatograph (Shimadzu Europe GmbH, D-47269 Duisburg, Germany) equipped with a BPX70 fused silica capillary column (SGE capillary column, length 30 m, i.d. 0.53 mm, 0.5 mm; 70% cyanopropyl polysilphenylene-siloxane stationary phase) and a flame ionization detector. The operating conditions were: initial temperature 75ºC, increasing by 4ºC/min to 148ºC; at 2.5ºC/min from 148 to 158ºC; and at 5ºC/min from 158 to 225ºC. The temperature of the injector and the detector remained stable at 280ºC. The column head pressure of the conductor gas (helium) was 1.30 g/cm^2. The FA percentage was integrated and calculated using the CLASS-Unipac Program (IZASA, C/Calàbria, 168–174, E-08015, Barcelona, Spain) by means of direct normalization of the peak areas. Each FA was identified in the form of a methyl ester by comparing the retention times with the corresponding standard (Sigma, St. Louis, MO 63103).
Data were statistically analyzed by analysis of variance and regression using the procedures described by the SAS Institute (1996) and GraphPad Prism(2002). When significant differences between treatments were detected ($P < 0.05$) means were compared by using the least significant difference method.

Results and discussion

Performance parameters were not significantly affected by dietary treatment. Average mean values were 2146 g for final BW (P=0.45); 136 g for daily FC from 21-42d old (P=0.27); 1.85 for Feed Conversion Ratio in this period (P=0.87).
Although total fat content of breast meat did not reach statistical significance (P=0.06), numerical differences were found between treatments (from 1.41% to 2.33% for the Control diet and 1% LO + 0.4% Capsomega diet respectively). However, no trend was detected with increasing content of LO or Capsomega in the diet: these results confirm that the replacement of variable ingredients in the experimental diets were adequate;

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previous experiments detected differences in performance parameters when tallow was replaced by LO (López-Ferrer et al., 2001b).
Linolenic, DHA and n-3 FA content in breast meat was higher with increasing levels in the diet (response could be approximated by linear regression; Figure 1). However, the response of DHA varied depending on the LO content of the diet: it was not affected when diet contained 1% LO, and it significantly increased when diet contained 2% LO (response could also be approximated by linear regression vs DHA or Capsomega content in diet; Figure 2). Some individual (animal) differences were observed between FA content of breast meat, that partly explained the different values obtained for 1% and 2% LO diets, as higher content of DHA in breast meat would be expected increasing the content of this FA in the diet as shown with 2% LO diets.
The ratio n-6 to n-3 FA in breast meat was 7.8 in soybean oil diet fed animals and 2.0 in 2% LO + 0.4% Capsomega fed animals; this represents a 3.9-fold variation not so far from the 5-fold variation found in previous experiments (López-Ferrer et al., 2001b). However, in the present experiment, LO + Capsomega represented only 2.4% at most, compared to 4% LO inclusion in the previous experiment.
Total n-3 FA content varied from 123 to 412 mg/200 g ration of breast meat, and DHA from 23 to 96 mg/200 g ration of breast meat in soybean oil diet fed animals and 2% LO + 0.4% Capsomega fed animals respectively. As a result of the n-3 FA and specifically DHA enrichment, this chicken meat would respond to the demands of the health conscious consumers (Hargis and Van Elswyk, 1993). Meat sensory tests (data not shown) confirmed that there was no incidence of unacceptable odours or flavours in breast meat. Therefore, the combination of LO and Capsomega in grower-finisher chicken diets is an interesting option to enrich meat in n-3 FA, and specifically DHA, without any impairment in broiler performance or meat sensory qualities.

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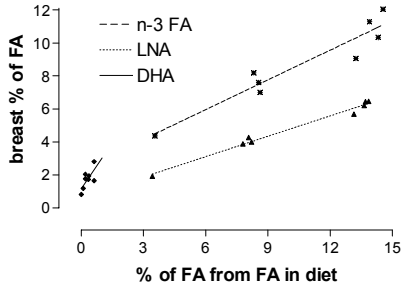
Table 1. Percentage of variable ingredients in feed treatments (21-42d of age)

Treatment	soybean meal	soybean oil	linseed oil	Capsomega[1]
1 (control)	0.200	2.200	0.	0.
2	0.150	1.150	1.000	0.100
3	0.100	1.100	1.000	0.200
4	0.	1.000	1.000	0.400
5	0.175	0.175	2.000	0.050
6	0.150	0.150	2.000	0.100
7	0.100	0.100	2.000	0.200
8	0.	0.	2.000	0.400

[1] Capsomega is a dried algae containing 20% DHA w/w

Figure 1. Linolenic, DHA and n-3 FA content of breast meat and feed (as % of FA).

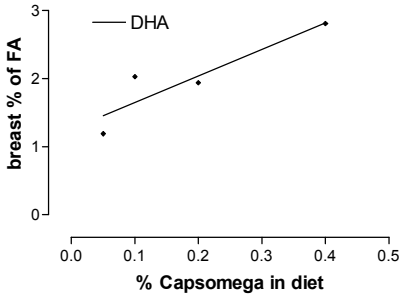


% LNA breast = 0.65 + 0.41 x % LNA feed
P<0.001 R^2=0.985 SEE=0.217

% DHA breast = 1.17 + 1.85 x % DHA feed
P<0.05 R^2=0.532 SEE=0.435

% n-3 breast = 2.36 + 0.60 x % n-3 feed
P<0.001 R^2=0.904 SEE=0.838

Figure 2. DHA content of breast meat (as % of FA) and Capsomega inclusion in feed (only for 2% LO treatments).



% DHA breast = 1.26 + 3.90 x % Capsomega
P=0.089 R^2=0.829 SEE=0.335

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EFFECT OF DIETARY FATTY ACID COMPOSITION ON CARCASS QUALITY OF BROILER

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Keywords : fat quality, diet, carcass, poultry

Abstract

Since the banning of animal products from poultry feed, the oily fat problem has been exacerbated by the exclusive use of vegetable oils and, from now, should be taken into account when formulating feed. The synthesis of five experiments has made possible to relate the fatty acid profile of the abdominal fat and dietary fats with the carcass presentation. The abdominal fat fatty acid profile was highly correlated with the lipids found in the diet. The more unsaturated the fats, the softer and oilier carcasses seemed. Polyunsaturated fatty acid contents accounted well for the visual scoring of carcasses ($r^2 = 0.64$, ETR = 0.50, n = 27).

Résumé

Depuis l'interdiction des produits animaux pour l'alimentation des volailles, le problème de gras huileux est exacerbé par l'utilisation exclusive des huiles végétales, et doit être dorénavant pris en compte dans la formulation des aliments. La compilation de cinq expérimentations a permis de relier les profils en acides gras du gras abdominal et des lipides alimentaires à la présentation de la carcasse. Le profil en acides gras de la graisse abdominale est bien corrélé à celui des lipides présents dans l'aliment. La notation visuelle des carcasses est bien corrélée aux teneurs en acides gras polyinsaturés des régimes (r^2= 0,64, ETR = 0,50, n=27).

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Introduction

In broiler chickens, the oily fat problem encountered in slaughterhouses causes technological incidents. Today, this problem is exacerbated by the exclusive use of vegetable oils resulting from the recent banning of animal fats and should be taken into account, from now, when formulating feed. Generally, the fatty acid profile of chickens' tissues reflects the composition of the ingested lipids. Significant correlations between the nature of dietary lipids and that of fatty and muscle tissues have been demonstrated, particularly for unsaturated fatty acids (Pinchasov et Nir, 1992 ; Caudron et al., 1993 ; Scaife et al., 1994). The main effect of an unsaturated dietary fat or oil is to induce the deposition, in body lipids, of polyunsaturated fatty acids that are not synthesized by chickens, namely linoleic and linolenic acids in fatty and muscle tissues, and long-chain polyunsaturated fatty acids in muscles (Lessire, 1995). Changes in the fatty acid profile of fat deposits in chickens result in a modification of the carcasses' aspect. Carcasses scores indicate they are firmer and drier when feeds are supplemented with tallow (Edwards et al., 1973; Caudron et al., 1993). Conversely, chickens fed on unsaturated oils show carcasses with softer fats (Caudron et al., 1993).

As part of the ACTA-ICTA program, "Dietary lipids and quality of fatty and muscle tissues in broiler chickens", a series of experiments showed that the composition of the fatty acids incorporated in the muscle triglycerides and phospholipids is closely linked to that of dietary lipids (Gandemer et al., 1999 ; Viau et al., 1999). The increase in n-3 PUFAs in intramuscular lipids has low impact on their sensitivity to oxidation. This good oxidation stability allows the use of diets with high linoleic acid proportions (7% of total fatty acids) with no impairment of chicken organoleptic qualities (Viau et al., 2001). The work presented here aims to define feed composition criteria in order to obtain an acceptable presentation of chicken carcasses at the slaughterhouse.

Material and Methods

Five successive experiments were carried out with a view to establish correlations between the fatty acid profile of the feed distributed to birds from day 22 to slaughter and the abdominal fat profile and visual quality of the carcass, following the distribution described in Table 1.

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Feeds

The same starter feed (with a 5% corn oil content) was given to all birds, followed by a feed containing 8% of various fats: tallow, palm and copra oils, sunflower, oleic sunflower, corn and linseed (Table 2). Feeds were manufactured at the "Station de Recherches Avicoles (INRA)" for experiments 1, 4 and 5, and in Sourches (Cybelia) for experiments 2 and 3.

For experiment 1, eight experimental diets were distributed, each characterized by its fatty acid profile: A, B, C, D, E, F, G, and H (Table 3). The saturated/unsaturated fatty acid ratio varied in the first 7 treatments with the modification of the relative proportions of the two main dietary polyunsaturated fatty acids: linolenic acid, which varied from 2 to 10% and linoleic acid, which was kept to 40% (treatments A to D). Linoleic acid varied from 20 to 60% in substitution for oleic acid and linolenic acid was maintained to 2% (treatments E to G). A tallow-based diet, supplemented with saturated fatty acids and depleted in linoleic acid (15%), was used as a reference (H).

The feeds tested for the following experiments are listed in Table 2. Additional diets were introduced to obtain intermediate profiles (I, M, J and K), which were slightly less saturated than H and reflected more the practice (prior to November 14, 2000). In the last experiment, C16:0 intakes were maximized with the use of palm and copra oils (O, P, Q and R).

Monitoring and measures

Birds were weighed collectively by pen at day 21 and individually before slaughter. Feed consumption was monitored by pen.

The various fatty acid profiles were determined by ITERG (Pessac) for oils, feed and abdominal fat mixtures sampled on 20 birds per treatment according standardized methods.

Carcasses were systematically scored by several trained persons, from 1 (soft and oily fat) to 5 (firm and dry fat).

A linear regression step by step was carried out to explain the carcasses score according to the polyunsaturated fatty acid contents.

Results and discussion

Growth performances barely changed with the nature of the lipids ingested. The abdominal fat percentages did not show significant variations between treatments.

The abdominal fat fatty acid profiles was highly correlated with the lipids found in the feed, particularly with C16:0 ($r = 0.97$), C18:2 ($r = 0.98$) and C18:3 ($r = 0.97$), and to a lesser extent with C18:0 ($r = 0.92$) and C18:1 ($r = 0.92$) (Figures 1 to 3).

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The carcass presentation score was correlated with the C16:0 content (r = 0.78) and with total polyunsaturated fatty acids (C18 :1 + C18 :2 + C18 :3) (r = -0,78). The more unsaturated the fats, the softer and oilier carcasses seemed. Therefore, polyunsaturated fatty acid contents in the feed account for the carcass scoring:

Carcass score = 5.756 - 0.11 C18:3 (%) - 0.04 C18:2 (%) - 0.03 x C18:1 (%)

r= 0.80, ETR = 0.50, n=27

The carcass scoring also depended on a polyunsaturated fatty acid synthetic variable (PUFA = C18:1 + C18:2 + C18:3):

Carcass score = 5.812 – 0.039 PUFA (%)

r= 0.78, ETR = 0.50, n=27

This evaluation was approximately equivalent. However, this equation gave less importance to C18:3 and did not take into account the proportion of polyunsaturated fatty acids between them. In order to obtain a proper carcass presentation (score > 3, moderately firm non-dry fat), the polyunsaturated fatty acid content should not exceed 72% of the dietary fat.

Conclusion

Thus, it is possible to use such equations when formulating feed in order to take into account the carcasses' visual and technological quality. The current practice, which consists in using 1 to 2% of palm oil in the feed, permits to meet these criteria and obtain good carcass presentations while maintaining a body fatty acid profile relatively unsaturated, therefore beneficial for the consumer.

TABLE 1: Experimental designs

Experiment	Location	Number of birds	Number of treatments	Age at slaughter (days)
1	INRA Magneraud	2064	8	42 to 49
2	Primex	1000	6	40
3	Cybelia	1664	7	42
4	INRA Magneraud	2400	8	42
5	INRA Magneraud	1200	6	42

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TABLE 2: Composition and characteristics of diets

Composition (%)	Starter feed (1 – 20 days of age)	Grower feed (21 days of age at slaughter)
Wheat	10	50
Corn	46	6,26
Soybean meal	31	32
Corn gluten	3,55	
Corn oil	5	
Added fat (*)		8
Lysine HCl	0,18	
DL Methionine	0,13	0,10
$CaCO_3$	1,32	1,18
Dibasic calcium phosphate	1,97	1,46
Mineral and Vit. mix	1	1
Characteristics		
Metabolizable energy (Kcal/kg)	3100	3150
Crude protein (%)	21,40	20
Lysine (%)	1,19	1,08
Methionine + Cystine (%)	0,86	0,77
Tryptophan (%)	0,24	0,25
Ca (%)	1	0,90
available P (%)	0,42	0,38

* Composition varied depending on the diets tested

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TABLE 3: Added fat composition of experimental diets *(%)

Diet	Experiment	Sunflower oil	Oleic sunflower oil	Linseed oil	Corn oil	Beef tallow	Palm oil	Copra oil
A	1, 2, 3, 4, 5	45.94	52.50	1.56				
B	1, 2, 3	44.75	46.75	8.50				
C	1, 2, 3, 4	42.78	42.22	15.00				
D	1	42.5	38.50	19.00				
E	1, 2, 3, 4		98.13	1.88				
F	1	23.25	75.00	1.75				
G	1, 2, 3, 4, 5	92.50	5.88	1.63				
H	1, 2, 3					100.00		
M	4		55.0	1.10	20.90	11.00		
I	3, 4		43.17	0.83		44.00		
J	4	11.30	11.14	3.96		61.60		
K	4	26.40	26.00	9.20		26.40		
O	5						100.00	
P	5						50.00	50.00
Q	5			12.50			87.50	
R	5		50.00				50.00	

** In experiment 2, the oleic sunflower oil was replaced by palm oil and an acidic vegetable oil.*

FIGURE 1

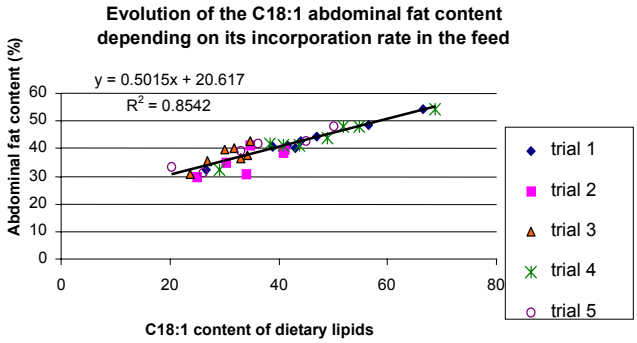

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FIGURE 2

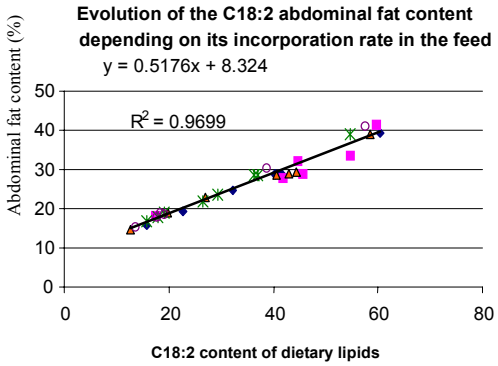
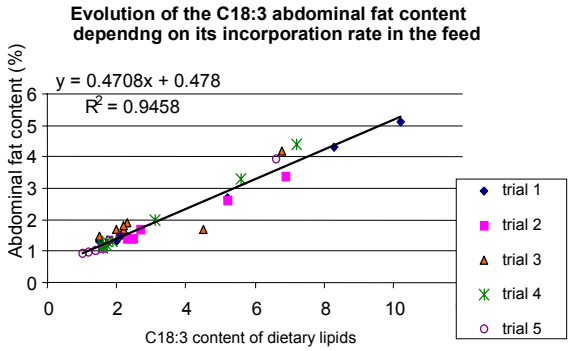


FIGURE 3



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EFFECT OF THE INCLUSION TIME OF EXTRUDED LINSEED SUPPPLEMENTATION BEFORE SLAUGHTER ON n-3 FATTY ACIDS ENRICHMENT OF CHICKEN MEAT

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Keywords : broiler chicken, extruded linseed, n-3 fatty acids, meat quality

Abstract

An experiment was conducted on nine hundred chicken of a label strain to study the effect of the inclusion time of extruded linseed on fatty acids (FA) composition and quality parameters of meat. The results showed that n-3 fatty acids content of thigh muscle increased with linseed supplementation and was related to the inclusion time of this supplement (27 *vs* 14 d before slaughter). This higher content of n-3 fatty acids resulted in a decreased n-6/n-3 ratio, which was beneficial for the consumers health. The treatments showed no effect on feed conversion and daily weight gain or carcass yield. No significant difference between meat quality parameters of the breast samples were detected, except for dry matter.

Résumé

Une expérience a été réalisée sur 900 poulets de souche à croissance lente pour étudier l'influence de la durée d'incorporation de graines de lin extrudées avant abattage sur la composition de la graisse intramusculaire et sur la qualité de la viande. Une augmentation significative de la proportion en acides gras n-3 est observée avec la

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complémentation en lin, la hausse la plus forte étant obtenue avec la durée d'incorporation la plus longue (27 j *vs* 14 j avant abattage). Le gain moyen quotidien, l'indice de consommation et le rendement carcasse sont similaires entre les différents régimes. De même, aucune différence significative de la qualité de la viande sur la base des paramètres étudiés n'a été observée, à l'exception de la matière sèche.

Introduction

In Western societies an unbalanced dietary consumption is observed, in particular an excess of saturated fatty acids is consumed and a too high n-6/n-3 polyunsaturated fatty acids ratio is noticed. Numerous research activities have been devoted to increasing the levels of n-3 polyunsaturated fatty acids, having beneficial effects on human health, in widely consumed products of animal origin whose lipid composition is easily modified. The use of linseed is a natural way that could reach this objective. The purpose of this experiment was to study the effect of the inclusion time of extruded linseed on the fatty acids pattern and quality of broiler meat.

Material and Methods

Animals and diets

Nine hundred males, 2-d-old chicks of a "label" strain were randomly arranged in 6 replicates (150 animals per pen) and three dietary treatments (2 replicates per treatment). Feed and water were provided ad libitum. The experimental diets, composed of wheat, maize and soybean meal, were formulated to be isoenergetic and isonitrogenous (Table 1). A part of wheat and soybean meal of control diet was replaced by extruded linseed (3.25 % of the diet) in the experimental diet. The three treatment groups were established according to the feeding program for the period corresponding to 46 to 73 d of age : birds were fed with control diet throughout the feeding period (R0) ; birds were fed with control diet from 46 to 59 d of age and with linseed diet from 59 to 73 d of age (R14) ; and birds were fed with linseed diet throughout the experimental period (R27). Feed conversion and daily weight gain were recorded at the end of the experimental period. Five animals of each pen were taken for further analyses (ten birds per treatment).

Laboratory analyses

Samples of each diet were analysed for nitrogen content, crude protein, dry matter, and lipid content. The fatty acids pattern of meat, performed on the broiler thigh muscle, was determined by difference using the Folch extraction method. Extracted lipids were

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saponified with KOH before to be methylated with 3% methanolic HCl to obtain fatty acid methyl esters (FAMEs). FAMEs were analysed for fatty acids pattern by using gas chromatography and internal standards. Parameters of meat quality were determined in each left breast of ten individuals per treatment. Cooking losses were determined by difference of weight of all the left breast of the samples, before and after cooking, wrapped in vacuum bags, in a Bain-marie at 75°c for 1 hour. Tenderness was calculated on cooked samples of breast (square portion of 1 cm in side, prepared in accordance with Touraille, 1981).
The texture of samples was determined by measuring the maximum shear force (N) with the Warner-Brazler shear tool adapted to a SMS-TAXT-2 Texture Analyser. The corresponding area (N*s) represented the hardness of meat.

Table 1: Calculated nutrient content, chemical analyses and major fatty acids composition of control and experimental diets enriched with extruded linseed

	Control diet	**Linseed diet**
Calculated nutrient content		
ME, Kcal/kg	2800	2800
Calcium, g/kg	9.50	8.88
Available P, g/kg	4.10	3.83
Methionine + Cysteine, g/kg	8.07	7.60
Lysine, g/kg	11.08	10.37
Chemical analyses	***%***	***%***
Dry matter	89.78	88.02
Crude protein	20.37	20.24
Ether extract	6.02	6.80
Crude fiber	2.49	2.83
Fatty acids pattern (g/100g fatty acids)		
C16:0	13.02	11.22
C18:0	2.80	2.80
C18:1	22.90	21.60
C18:2	55.83	47.68
C18:3	4.72	15.90
Σ SFA	16.46	14.74
Σ MUFA	22.99	21.68
Σ PUFA	60.55	63.58
n-6/n-3	11.84	3.00

SFA = saturated fatty acids ; MUFA = monounsaturated FA ;
PUFA = polyunsaturated FA

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Statistical analysis

Analysis of variance (ANOVA) was used to compare the three treatments. The homoscedasticity and normality hypotheses were respectively checked with Bartletts's test and with Ryan and Joiner's test. Some parameters that did not respect one or both applications conditions were submitted to logarithmic transformation before retesting the two hypotheses and performing one-way ANOVA. When ANOVA pointed out some significant differences between the three treatments, the mean observed in the three groups were compared with Tukey's pairwise comparison test (family error rate = 0,05).

Results and discussion

As expected, only the different n-3 fatty acids content of the dietary fat gave rise to a typical difference in the pattern of the thigh fat of birds receiving control or experimental treatments (Table 2). There were no significant difference in the total saturated, monounsaturated and polyunsaturated fatty acids contents between treatments because of similar proportion of these FA classes in the two diets (Table 1). According to Lopez-Ferrer et al. (2001), the higher content of n-6/n-3 ratio, which tried to achieve human health recommendations

Table 2 : Fatty acids composition of thigh samples of chickens assigned to three different feeding programs

Fatty acid (g/100g FA)	**R0**	**R14**	**R27**	**P**
C16:0	22.38 [1.54]	22.54 [0.97]	21.83 [0.90]	NS
C16:1 n-7	3.62 [0.73]	3.96 [0.99]	3.87 [1.02]	NS
C18:0	9.00 [0.65]	8.43 [0.69]	8.69 [0.77]	NS
C18:1cis n-9	23.98 [2.50]	24.59 [2.25]	25.58 [3.29]	NS
C18:2cis n-6	25.61 [3.43]	24.79 [1.55]	23.89 [2.49]	NS
CLA	0.38 [0.09]	0.35 [0.06]	0.34 [0.10]	NS
C18:3 n-3	1.74 [0.36]	2.93 [0.32]	3.80 [0.51]	***
C20:3 n-9	0.59 [0.09]	0.56 [0.12]	0.55 [0.10]	NS
C20:3 n-6	0.34 [0.06]	0.28 [0.06]	0.38 [0.13]	NS
C20:4 n-6	4.98 [0.75]	4.53 [0.94]	4.32 [0.98]	NS
Σ SFA	34.38 [3.40]	33.97 [1.87]	33.57 [2.17]	NS
Σ MUFA	31.74 [2.75]	32.36 [2.42]	32.97 [3.69]	NS
Σ PUFA	33.88 [3.77]	33.66 [2.15]	33.48 [3.66]	NS
n-6/n-3	18.54[a] [3.43]	10.28[b] [1.20]	7.63[c] [064]	***

[a,b,c]Values in the same row with no common superscript are significantly different
SFA = saturated fatty acids ; MUFA = monounsaturated FA ; PUFA = polyunsaturated FA
[ ] = standard deviation
***P< 0.001

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Figure 1 showed a positive effect of linseed including time on C18:3 n-3 content of thigh fat. The linear response observed seemed to indicate a constant pattern of change in C18:3 n-3 accumulation throughout the experimental period.

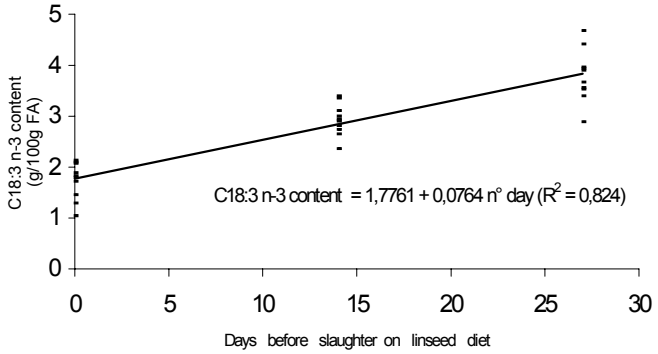

Figure 1 : Influence of the number of days prior to slaughter in which birds received linseed supplement on C18:3 n-3 content of thigh fat

There were no significant difference in performance parameters among treatments excepted for final weight. However, the slightly higher weight of R27 birds couldn't be attributed to the feeding program because this little difference was already observed in the weight of birds at the beginning of the trial period. The linseed supplementation tended to improve feed efficiency, although there was no significant difference.

Table 3 : Performance parameters of chicken

Variable	**R0**	**R14**	**R27**	**P**
Weight at 2 d (g per bird)	39.58 [1.22]	39.49 [1.30]	39.48 [1.21]	NS
Weight at 46 d (kg per bird)	1,57[a] [0.20]	1,57[a] [0.19]	1.61[b] [0.18]	*
Weight at 73 d (kg per bird)	2.92[a] [0.30]	2.97[a] [0.27]	3.05[b] [0.30]	*
Daily weight gain, g per bird between 46 and 73 d	50.27 [0.14]	51.97 [1.76]	53.4 [0.86]	NS
Feed efficiency between 46 and 73 d	3.15 [0.15]	2.94 [0.11]	2.93 [0.06]	NS
Carcass yield at slaughtering (%)	65.69 [4.22]	64.15 [5.53]	62.66 [4.76]	NS

[a,b]Values in the same row with no common superscript are significantly different
[ ] = standard deviation
*P< 0.05

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No differences of meat quality parameters between treatments were detected, excepted for dry matter (Table 4). We had no explanation for this observed difference.
Although analysis of variance revealed no significant difference, the thigh fat content tended to gradually increase with the linseed including time. This observation had no effect on tenderness and cooking losses parameters.

Table 4 : Quality meat parameters

Variable	R0	R14	R27	P
Dry matter (%)	23.59[a]	24.27[b]	23.77[ab]	*
	[0.42]	[0.67]	[0.57]	
Fat (% DM)	7.30	7.60	8.18	NS
	[1.19]	[1.48]	[1.53]	
Tenderness :				
Force (N)	14.54	14.47	14.75	NS
	[2.24]	[2.72]	[1.88]	
Hardness (N*s)	135.15	133.46	146.76	NS
	[21.10]	[27.22]	[22.41]	
Cooking losses (%)	16.75	16.43	16.89	NS
	[1.86]	[2.53]	[1.79]	

[a,b]Values in the same row with no common superscript are significantly different
[ ] = standard deviation
*P< 0.05

However, the slaughterer, who didn't know the trial protocol, had given his opinion about carcass quality at the slaughtering time. He immediately picked the two pens who had received the linseed supplement during the longer time out in the 6 pens (150 birds per pen). According to him, the birds of these two pens were plucked easier and had a nicer and more homogeneous visual quality carcass that those of the other pens.

Acknowledgements

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QUALITY TRAITS AND OXIDATIVE STABILITY OF N-3 PUFA ENRICHED CHICKEN MEAT

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Keywords: broiler chicken, n-3 PUFA, oxidative stability, meat and carcass quality

Abstract

The aim of this study was to evaluate the effects of dietary addition of different doses of dehydrated algae on oxidative stability, colour, water holding capacity and sensory properties of chicken meat. A total of 360 female Cobb 500 received from 21 d to slaughtering age a corn-soybean commercial diet plus 0.5% (ALGA0.5) or 1% (ALGA1) *Schizochitrium sp*. algae. Control group was fed the commercial diet.
The iron ascorbate induced lipid oxidation analysis of breast meat samples showed a significantly lower stability in group ALGA1 compared to Control. A similar trend was also observed in drumstick meat. The higher dietary supplementation level of alga significantly affected breast meat colour by enhancing the values of lightness (L*), redness (a*) and yellowness (b*). Water holding capacity of breast and organoleptic traits of drumstick meat were insensitive to the dietary treatment.

Résumé

L'objectif de cette étude était d'évaluer les effets d'une supplémentation alimentaire en différentes doses d'algues déshydratées sur la stabilité oxydative, la couleur, la capacité de rétention en eau et les propriétés sensorielles de la viande de poulet. Un total de 360 femelles Cobb 500 recevait un aliment commercial à base de soja et maïs avec l'addition de 0.5% (ALGA0.5) ou 1% (ALGA1) de algae *Schizochitrium sp*. de l'âge de 21 jours jusqu'à l'abattage. Le lot témoin était nourri avec l'aliment commercial non supplémenté. L'analyse de l'oxydation des lipides induite par le fer ascorbate sur les échantillons de filet, montre une stabilité significativement inférieure dans le groupe ALGA1 comparée à celle du témoin. Ce résultat a été obtenu également dans les échantillons de pilons de poulet. La couleur du filet a été significativement affectée seulement par l'addition de la plus haute quantité d'algues qui accroît les valeurs de luminosité (L*) et d'intensité de rouge (a*) et de jaune (b*).

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La capacité de rétention en eau des filets et la qualité sensorielle des pilons n'ont pas été affectées par le traitement alimentaire.

Introduction

Considering the beneficial effects of n-3 polyunsaturated fatty acids (PUFA) for human health (Leskanich and Noble, 1997), several studies have been carried out to enrich animal products with these compounds. Both eggs and poultry meat enriched with n-3 PUFA may be considered as valid n-3 PUFA alternative sources to natural occurring fish products. To fortify poultry meat, different n-3 PUFA sources, such as fish meal and oil, linseed, canola and marine algae have been used (Hulan et al., 1988, Abril and Barclay, 1998, Gonzalez-Esquerra and Leeson, 2001, Lopez-Ferrer et al. 2001, Sirri *et al.,* 2002). However n-3 PUFA, due to their double bonds, are highly sensitive to oxidative deterioration and responsible for the formation of peroxides and off-flavours and taste deterioration (Eriksson, 1987, Gonzalez-Esquerra and Leeson, 2000, 2001). No information are available in literature about the oxidative stability of meat of broiler fed diets added with marine algae as n-3 PUFA source. The aim of this study was to evaluate the susceptibility to lipid oxidation of breast and drumstick muscles, the colour of different carcass tissues, and the sensory properties of drumstick meat in broiler chickens fed diets cointaining different levels of the *Schizochitrium* sp. marine algae.

Material and Methods

A total of 360 day old female Cobb 500 reared on 18 litter pens (60 birds/pen), received from 21 d to slaughter (46 d) a corn-soybean commercial diet added with 0.5% or 1% the *Schizochitrium* sp. algae, a marine source of n-3 PUFA which contains about 20% docosahexaenoic acid (DHA) (ALGA0.5 and ALGA1 respectively). The control group (Control) had no supplementation. Twelve breasts and drumsticks per group were collected at slaughter and stored at –40°C until analysis. Lipids from breast and drumstick were extracted by Folch method (Folch *et al*., 1957) and the ether extract was divided into 2 aliquots and used either for the total lipids determination or for the fatty acid analysis. Fatty acids were converted to methyl esters following the Christopherson and Glass method (1969). The separation of fatty acids was carried out by using a Shimadzu GC17A gaschromatograph with a WP-4 Shimadzu integration system, equipped with a Supelco SP2340 capillary column and a flame ionization detector (FID). Iron-ascorbate-induced lipid oxidation was measured according to the method described by Kornburst and Mavis (1980). Colour of breast, thigh, skin and fat

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pad was evaluated by using the CIE L* a* b* system with a Minolta Colorimeter CR 300. Water holding capacity was determined for drip loss, whereby the loss of water from slice of breast muscle weighing 50 g kept for 24 h suspended in glass box at 4 C was registered (Honikel, 1998). To evaluate differences in flavour and odour among groups triangular test was performed (Porretta, 1992): 20 g of drumstick were singularly packed in aluminium foil, oven cooked at 100°C for 20 min and tasted by 30 untrained panellists at a temperature of about 50°C. Data were submitted to one-way ANOVA using the General Linear Model of SAS. Differences among groups were separated by Student Newman Keuls test.

Results and discussion

The lipid content of both breast and drumstick was not affected by the lipid supplementation of the diet (Table 1). Enhancing the dietary algae dose, the breast n-3 PUFA content, expressed as mg/g of lipids, significantly increased and the n-6 PUFA content decreased. Moreover n-6/n-3 ratio was significantly reduced by the algae supplementation. In drumstick the same trend of breast was observed but the difference among groups were not statistically significant. In breast the total content of lipids is consistently lower than that of drumstick and mainly represented by intramuscular lipids such as cell membrane phospholipids which are notoriously rich in long-chain n-3 PUFA (Leskanich and Noble, 1997). Drumstick being characterised by both intramuscular and intermuscular lipids contains much more triglycerides, whose n-3 PUFA content is consistently lower than that of phospholipids. This could explain the major effect of algae supplementation in breast in comparison with drumstick meat (Table 1).

The lipid oxidation analysis of breast meat samples showed that the production of malondialdehyde (MDA) increased as the incubation time increased. Breast meat from ALGA1 birds had significantly higher MDA concentrations with values double of those of Control ones (Table 2). A similar trend was observed in drumstick samples from ALGA1 but the differences were less remarkable and not significant even if the figures were 30% higher than Control (Table 3). MDA concentration was considerably higher in drumstick than in breast meat due to its higher lipids content (2.6 *vs.* 1.1%). The large difference between Control and ALGA1, observed in breast, is explained by the higher proportion of n-3 PUFA (44.2 *vs*. 16.4 mg/g of lipids respectively for ALGA1 and Control).

Algae supplementation significantly affected breast meat colour enhancing the values of lightness (L*), redness (a*) and yellowness (b*), particularly in group ALGA1. On the contrary, no effect was observed in skin and thigh meat with the exception of

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yellowness (b*) which was slightly lower in thigh of ALGA0.5. Yellowness of abdominal fat pad increased in groups supplemented with algae (Table 4).
Water holding capacity of breast meat was not affected by the dietary treatment. As for organoleptic evaluation, panellists were not able to differentiate the meat samples of treated birds from those of controls (Table 5).
The use of algae in feed formulation of chicken broiler, while improving the nutritional value of meat due to their content of n-3 PUFA, and enhancing the colour intensity of meat, does not alter the organoleptic properties. The incorporation of highly unsaturated fatty acids, however, leads to a reduced lipid stability of muscles, particularly of breast, that might be prevented by enhancing the level of antioxidants in feed.

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Table 1. Total lipids (%) and fatty acid content of drumstick and breast (mg/g fat)

N = 20	Control	ALGA0.5	ALGA1	SEM
Breast				
Total lipids	1.27	1.15	1.11	0.003
Total saturated	222.23	215.10	216.08	22.52
Total monounsaturated	190.74	170.73	168.14	16.31
20:4 n-6	39.63	42.70	37.80	2.35
20:5 n-3	1.34 Bb	2.77 ABb	3.16 Aa	0.36
22:5 n-3	5.17	5.94	5.90	0.54
22:6 n-3	4.12 Bc	22.95 Ab	33.69 Aa	2.65
Total polyunsaturated	263.14	261.78	239.85	18.58
Total n-3	21.61 Bc	40.55 Ab	50.10 Aa	2.85
Total n-6	231.34	215.04	184.03	24.20
n-6/n-3	10.71 A	5.30 B	3.67 C	1.43
Drumstick				
Total lipids	2.52	2.65	2.62	0.006
Total saturated	237.27	200.78	192.00	15.57
Total monounsaturated	271.31	220.85	212.44	18.65
20:4 n-6	30.23	21.52	23.91	4.72
20:5 n-3	0.98 Bb	0.41 aBb	2.00 Aa	0.14
22:5 n-3	3.72	3.14	3.39	0.32
22:6 n-3	2.45 Bc	10.81 Ab	15.84 Aa	1.19
Total polyunsaturated	339.49	263.55	261.92	25.44
Total n-3	25.95	29.16	34.76	2.55
Total n-6	309.33	231.12	224.13	22.92
n-6/n-3	11.92 A	7.95 B	6.51 C	0.28

Table 2. Effects of the dietary supplementation of dehydrated algae on induced lipid oxidation of breast chicken meat (nmol MDA/mg protein).

Incubation time	Groups (n = 20)			
(min)	Control	ALGA0.5	ALGA1	SEM
0	0.417 B	0.528 AB	0.680 A	0.057
30	0.500 b	0.735 ab	0.932 a	0.101
60	0.538 b	0.760 ab	0.960 a	0.106
90	0.524 B	0.810 AB	1.094 A	0.120
120	0.511 b	0.850 ab	1.063 a	0.128
150	0.632	0.880	1.084	0.135

a, b: P< 0.05; A, B: P < 0.01.

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Table 3. Effects of the dietary supplementation of dehydrated algae on induced lipid oxidation of drumstick chicken meat (nmol MDA/mg protein).

Incubation time	Groups (n = 20)			
(min)	Control	ALGA0.5	ALGA1	SEM
0	0.713	0.694	0.819	0.075
30	0.881	0.841	1.177	0.160
60	1.048	1.035	1.405	0.194
90	1.077	1.209	1.462	0.225
120	1.155	1.246	1.496	0.224
150	1.193	1.239	1.662	0.239

Table 4. Effects of the dietary supplementation of dehydrated algae on breast, thigh, skin and abdominal fat pad colour.

	Groups (n = 20)			
	Control	ALGA0.5	ALGA1	SEM
		Breast		
L*	48.50 Bb	49.86 ABb	52.13 Aa	0.77
a*	0.56 B	0.52 B	2.46 A	0.41
b*	7.80 B	8.35 B	11.37 A	0.51
		Thigh		
L*	53.35	53.65	52.09	0.50
a*	3.22	3.01	3.29	0.36
b*	8.47 a	6.69 b	7.61 ab	0.47
		Skin		
L*	73.85	70.58	72.73	1.07
a*	3.11	2.81	2.60	0.66
b*	24.54	24.75	24.81	0.69
		Abdominal fat pad		
L*	75.08	74.80	74.59	0.69
a*	-1.05	0.17	0.49	0.48
b*	26.59	29.29	30.07	1.27

a, b: $P < 0.05$; A, B: $P < 0.01$.

Table 5. Results of panel test of drumstick meat (triangular test).

N = 20	Panelists, n.	Positive responses	Probability
ALGA0.5	30	11	41.5 (not significant)
ALGA1	30	11	41.5 (not significant)

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TOTAL FATTY ACID QUANTIFICATION AS AN ESTIMATOR OF TOTAL BODY FAT CONTENT IN BROILERS FED UNSATURATED DIETS

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Keywords: broilers, PUFA, body fat, hydrolysed crude fat

Abstract

An experiment was designed to determine the effect of dietary fat unsaturation level on the fat content of the body of broilers, measured as hydrolysed crude fat (CF) and total fatty acid (TFA) content. Ninety-six female broiler chickens were fed with 4 experimental diets (with 15, 34, 45 and 61 g of polyunsaturated fatty acids (PUFA)/kg of diet) for 6 weeks. As dietary PUFA increased from 15 to 61 g PUFA/kg the fat content of the animals decrease was 55% and 15% when measured as CF and TFA content respectively. As we did not find any differences between treatments in crude protein (CP) and ashes (A) content, the total chemical composition (CF, CP and A as a percentage of Dry Matter (DM)) was close to 100% in the animals fed the saturated diets, reaching only 80% when the dietary unsaturation level was maximum. When using TFA instead of CF, the total chemical composition was close to 95% of DM in all treatments.

Résumé

Une expérience a été effectuée pour déterminer l'effet du niveau de la graisse insaturée dans l'aliment sur le contenu de la graisse corporelle des poulets mesuré par la teneur en graisse brute hydrolysée (CF) et par la teneur en acides gras totaux (TFA). Quatre-vingt seize femelles broilers ont été alimentés avec 4 régimes expérimentaux (contenant 15, 34, 45 ou 61 g d'acides gras polyinsaturés (PUFA)/kg d'aliment) pendant six semaines. Quand la teneur en PUFA du régime augmente de 15 à 61 g/kg l'engraissement des animaux diminue de 55% et 15% quand il est mesuré par la teneur en CF et TFA respectivement. Comme on n'a pas trouvé de différences entre les traitements pour la teneur en protéines brutes (CP) et en cendres (A), la composition totale chimique (CF, CP et A exprimés en pourcentages de la matière

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sèche (DM)) était près de 100% chez les animaux alimentés avec le régime riche en AG saturés, et seulement de 80% quand le niveau d'insaturation du régime était maximal. En utilisant le TFA au lieu de CF, la composition totale chimique était près de 95 % de la DM pour les 4 traitements.

Introduction

It is usual to include fat in poultry diets as a source of energy. Increasing concern about safety of animal fats has led to a higher use of vegetable oils, rich in unsaturated fatty acids, in animal nutrition. These fatty acids are better absorbed (Wiseman and Salvador, 1991) and thus provide more metabolizable energy to the animal. Some studies suggest that, once absorbed, saturated and unsaturated fatty acids are not used in the same way. Shimomura et al (1990) showed that rats fed safflower oil had less body fat content due to increased thermogenesis and higher lipolitic activity than rats fed tallow. In poultry, some authors found no effect of dietary PUFA on fat deposition (Pinchasov and Nir, 1992) but others (Sanz et al., 1999) reported lower fat content in broilers fed unsaturated fats. This study was designed to asses the effect of dietary PUFA on the fat content of broilers, measured as CF and as TFA content.

Material and Methods

Animals and diets

The trial received approval from the Animal Protocol Review Committee of the UAB. Ninety-six female broiler chickens of 1 day of age were randomly distributed into 4 dietary treatments (12 replicates). The animals were housed in 48 cages under standard conditions of temperature, humidity and ventilation. Four levels of dietary PUFA (15, 34, 45 and 61 g of PUFA/kg) were achieved by blending different amounts of tallow, linseed and fish oil, keeping the added fat at 9%. Composition of the diets is shown in table 1. Feed and water were provided *ad libitum*. Body weight and food consumption were measured at days 8, 18 and 44 to determine average daily gain (ADG), average daily intake (ADI) and food conversion ratio (FCR).

Slaughter and sample collection

At 44 days of age the animals were killed by lethal injection. The whole bodies of the animals (including feathers and blood) were frozen and minced. A representative sample was taken, freeze-dried, ground and stored at -20ºC until further analysis.

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Chemical analysis

The DM, A, CP, crude fibre and CF content of diets were determined (AOAC, 1995). The fatty acid amount of the diets was quantified by gas chromatography (GC) using nonadecanoic acid as internal standard (Sukhija and Palmquist, 1988). Whole body samples were analysed for gross energy (GE) with an adiabatic bomb calorimeter (IKA calorimeter C-4000) and for CP, CF and ash content (AOAC, 1995). Samples were subjected to acidic digestion before CF analysis to cause disruption of lipid-protein binding (AOAC, 1995). The fatty acid content was quantified by GC using nonadecanoic acid as internal standard (Carrapiso et al., 2000).

Table 1: Composition and chemical analysis of the experimental diets

Ingredients	%	Chemical analysis	%
Wheat	39,30	Dry matter	90,78
Soya 48	34,09	Crude protein	22,98
Barley	13,39	Crude fat	10,17
Added fat[1]	9,00	Crude fibre	3,47
Bicalcium phosph.	2,17	Ash content	6,08
Calcium carbonate	0,98	Crude Energy (Kcal/Kg)	4481
Salt	0,45	Estimated values	
Vitamin-mineral mix[2]	0,40	Metabolizable Energy (Kcal/Kg)	3100
DL – methionine	0,28	Lysine (%)	1,200
L – Lysine	0,04	Methionine (%)	0,597

[1] 100 % tallow (15 g PUFA/kg); 60 % tallow – 40 % linseed and fish oil (34 g PUFA/kg); 40% tallow – 60 % linseed and fish oil (45 g PUFA/kg); 100 % linseed and fish oil (61g PUFA/kg).

[2]Vitamin and mineral mix per kg of feed: Vitamin A: 12000 UI; Vitamin D_3: 2400 UI; Vitamin K_3: 3 mg; Vitamin B_1:2.2 mg; Vitamin B_2: 8 mg; Vitamin B_6: 5 mg; Vitamin B_{12}: 11 □g; Folic acid: 1.5 mg; Biotin: 150 □g; Calcium pantotenate: 25 mg; Nicotinic acid : 65 mg; Mn: 60 mg; Zn: 40 mg; I: 0,33 mg; Fe: 80 mg; Cu: 8 mg; Se: 0.,5 mg.

Statistics

Data were analysed by ANOVA using the GLM procedure of SAS statistical package (SAS® Institute, 1996). Dietary PUFA was the factor included in the model. Differences between treatment means were tested using SNK test. The level of significance was pre-set at $P < 0.05$.

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Results and discussion

ADI and FCR (data not shown) were significantly affected by the dietary degree of unsaturation (P<0.05). As dietary PUFA increased, ADI decreased (from 111,72 to 104,56 g/day for the 15 and 61 g PUFA/kg diets respectively, P<0.05). There were no differences in ADG, thus FCR was worse for the animals fed with the more saturated diet (P<0.05). There were no differences between treatments on final body weight. Unsaturated fats had better digestibility and therefore, have a higher metabolizable energy concentration available for the animal. The lower consumption of the diets including this type of fat can be due to a regulation of feed intake depending on the dietary metabolizable energy (NRC, 1994).
The chemical composition of the whole animal is shown in table 2. CP and ash content were not affected by treatment. CF and GE were both affected negatively with dietary unsaturation, but while GE decrease was 10%, CF decrease was 55% when dietary PUFA increased from 15 to 61 g/kg of feed. Moreover, the sum of the three major components on a DM basis (CP, CF and A contents, as carbohydrate content of the whole body was negligible) was around 100% only for the animals fed with the saturated diets. This fact, added to the difference existing between GE and CF reduction, suggests that the CF content reduction due to dietary PUFA might be overestimating reality.

Table 2: chemical composition (% of DM) and gross energy content of the whole body of broilers (44 days of age).

	g PUFA/ kg of diet				P	RSD
	15	34	45	61		
Water %	62,55	61,63	62,73	63,16	0,282	2,605
CP	52,21	53,02	53,41	54,51	0,246	3,787
CF	40,75 [a]	38,21 [a]	33,30 [b]	18,23 [c]	**<0,001**	4,682
U:S[1]	2,02[a]	2,45[b]	2,67[c]	3,48[d]	**<0,001**	0,196
A	6,63	6,63	7,02	7,22	*0,091*	0,828
DM %[2]	99,63	97,86	93,73	79,96		
GE (Kcal/kg)	6510[a]	6318[b]	6223[b]	5857[c]	**<0,001**	221,5

[1] U:S is the ratio between unsaturated and saturated fats in the whole animal body.
[2] DM% is the sum of CP, CF and ash content on a dry matter basis.
[a,b,c] Means within a row with different superscript letters were statistically different (p<0.001).

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Using the TFA content as an estimator of the fat content, as shown in table 3, we can still see a reduction (15 %) in the body fattening as dietary PUFA raised from 15 to 61 g/kg. The total chemical composition (CP, TFA and ash content) was in all cases around 95%. TFA content did not include all the fat-soluble molecules in the body and did not include either the glycerol moiety of triglycerides.

Some authors have found a lower body fattening of broilers when fed PUFA-enriched diets. Thus, Sanz et al. (2000a) found higher body fat and energy content in broilers fed tallow than those fed on diets containing vegetable oils, and suggest that the unsaturation degree of dietary fat affects its metabolic use. In a posterior work, Sanz et al. (2000b) observed that broilers fed unsaturated fats show higher ß-oxidation and lower fatty acid synthesis. Crespo and Esteve-Garcia (2002a, 2002b), when studying the different fat depots of broilers fed different dietary fats, show that the weight of separable fat depots (like abdominal fat pad) is more reduced in animals fed sunflower and linseed oil than those fed tallow. However, these authors found a higher fatty acid synthesis in the animals fed unsaturated fats, and attribute the lower fat depots to a higher ß-oxidation rate.

Our work showed a reduction of the energy storage (mainly body fat) as the PUFA intake increased. Otherwise TFA can be an acceptable estimator of the whole body fat content, and is even more precise than hydrolised CF determination in the case of highly unsaturated meats.

Table 3: TFA content (% of DM) of the whole body of broilers (44 days of age).

	g PUFA/ kg of diet				P	RSD
	15	34	45	61		
TFA	37,48 [a]	35,09 [a]	34,94 [a]	31,82 [b]	**0,002**	4,674
Sum	96,32	94,74	95,37	93,55		

[1] DM% is the sum of CP, TFA and ash content on a dry matter basis.

[a,b] Means within a row with different superscript letters were statistically different ($p<0.001$).

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FATTY ACIDS PROFILE OF LIPIDS FROM DUCK MUSCLES OF THREE POLISH CONSERVATIVE FLOCKS

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Keywords: conservative flock, duck meat, lipids, fatty acids

Abstract

The aim of the study was the comparison of fatty acid profile of duck muscles from three conservative Polish flocks (A3, P33, K2). The unsaturated fatty acids (UFA) were predominant for all flocks (50.12-62.66%). The muscles of A3 birds contained the most of UFA. The highest level of polyunsaturated fatty acids (PUFA) in K2 muscles was established. The muscles of P33 birds contained the most of saturated fatty acids (SFA). The UFA/SFA ratio was 1.19-1.98.The PUFA/SFA ratio was 0.63-0.92, PUFA n-6/n-3 ratio was 3.85-5.85. The lipids from A3 muscles were characterized by the best value of the PUFA n-6/n-3 ratio (3.85.- breast, 4.57- leg muscles) and the best fatty acids profile.

Résumé

L'objectif de cette étude était de comparer la composition en acides gras des muscles de trois souches de canards sélectionnées en Pologne. Les acides gras insaturés (IAG) dominaient pour toutes les souches (50.12-62.66%). Les muscles des canards du groupe A3 en contenaient le plus. Les muscles des canards du groupe K2 contenaient le plus d'acides gras polyinsaturés (AGPI). Par contre, ceux des canards du groupe P33 contenaient le plus d'acides gras saturés (AGS). La proportion AGI/AGS était de 1.19-1.98; AGPI/AGS = 0.63-0.92 et AGPI n-6/n-3 était de 3.85-5.85. Les lipides des muscles des canards du groupe A3 se caractérisaient par la meilleure composition en acides gras et par la proportion AGPI n-6/n-3 la plus favorable.

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Introduction

During the last years nutritional literature at all levels was filled with arguments on the role of dietary fat in the increased incidence of the many ailments in particular the coronary heart disease and cancer. The general opinion is that the incidence of such disorders would be reduced and the health of our society improved by the reduction in the gross amount of fat consumed, a reduction in dietary cholesterol and a change in the regimen of fatty acids in favour of increased levels of a range of polyunsaturates (Leskanich and Noble 1999). It was assumed that fat in a suitable daily diet of a healthy human being should cover 23-25% of energy. Beside that this dose should cover full daily demand of a human organism for fat - soluble vitamins and essential unsaturated fatty acids. Moreover, the ratio of saturated (SFA) to unsaturated (UFA) fatty acids ought to be on the definite level. The PUFA/SFA ratio should be around 0.45 and intakes of n-3 PUFA should be increased relative to n-6 to bring the ratio PUFA n-6/n-3 below 4.0- 5.0 (Pikul 1996, Garcia and Casal 1999). At present there are tendencies to increase the level of monounsaturated fatty acids in human diets and to decrease the level of saturated fatty acids (Skrabka- Błotnicka et al. 1999).
The studies of the quality of ducks' meat confirmed differentiation between species, lines such as: body weight, meat and fat yield in the carcass. Besides, it is commonly known, that several genotypes and strains of ducks differ in nutritional value, sensory attributes and functional properties of meat. Therefore, the purpose of our study, was a comparison of fatty acids composition of lipids in duck muscles from three Polish conservative flocks.

Material and Methods

Ducks from three conservative groups of ducks maintained *in situ* method in the Department of Waterfowl Breeding Research National Institute of Animal Production in Kórnik near Poznań and specified below were used as experimental material: 1) the Mini- ducks (K2), bred from wild ducks (Anas platyrhynchos L.) and Pekin ducks (FAO, 2000), 2) Polish Pekin (P33), native breeding strain taken from the farm at Borowy Młyn (FAO, 2000). 3) duck population A3, progeny of a commercial stock imported from England in 1977.
Birds were fed *ad libitum* with a compound feed mixture containing up to 20% crude protein and up to 12.13MJ metabolize energy, given till the third week of age, and later up to 16.5% crude protein and 12.34 MJ metabolize energy per kg of feed.

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Breast and leg muscles (12 breast- B and leg- L muscles) used for the analysis were taken from six 7-week old drakes from each duck's group (having body weight closed to the arithmetic mean of sex in the given group).
The fatty acids composition was carried out using the gas chromatography. The AGILENT Tech. 6890N Chromatograph was used. The methyl esters of fatty acids were separated on the CP-Sil 88 (Chrompack) capillary column (100 x 0.25 mm), helium was used as the carrier gas. The separation was conducted at the programmed temperature from 165 to 200^{o}C by increase rate at 2^{o}C/min.
The results were subjected to statistical analysis comprising: elimination of gross errors and determination of the significant differences in the average values of experimental results. The statistical analysis was conducted with the application of the SCIENTIST PC program.

Results and Discussion

It was stated that in the fatty acids composition of lipids from muscles of all groups the $C_{18:1}$ and $C_{16:0}$ were predominant (table 1). The highest content of $C_{18:1}$ (22.07-33.72%) and $C_{16:0}$ (17.70-24.31%) was stated among the identified fatty acids. It was in accordance with data obtained by Turi et al. (1994), Romboli et al. (1997) for Muscovy ducks, Smith et al. (1993) for Pekin ducks and Wołoszyn (2001) for force fed Mulard ducks. The kind of muscles was affected on $C_{16:0}$ and $C_{18:1}$ content and species of ducks on $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, and $C_{20:4}$. There was determined in the breast muscles the higher content of $C_{16:0}$ and lower $C_{18:1}$ than in the leg muscles of all birds. It was established that in muscles of birds from A3 group was the lowest content of $C_{18:0}$ and $C_{18:2}$ and significantly more of $C_{18:1}$ in comparison with the remaining muscles. In the muscles of birds from K2 group was the highest amount of $C_{20:4}$.
The unsaturated fatty acids (UFA) were predominant in fatty acid composition for all flocks (50.12-62.66%). At the same time the leg muscles were characterized by higher level of the UFA (especially monounsaturated) than breast. The breast muscles contained more (38.16-42.04%) saturated fatty acids (SFA) than leg muscles (31.63-34.99%). The MUFA amounted to 23.46- 30.75% and the PUFA to 25.97- 30.44% of the global content of fatty acids. The highest level of the PUFA was established in K2 muscles. The data relative to the UFA and the MUFA contents agreed with the results previously published by Salichon et al. (1993), Romboli et al. (1997) for Muscovy and Smith et al. (1993), Leskanich and Noble (1997) for Pekin ducks. However, the muscles from the investigated ducks were characterized by the significantly higher PUFA content in comparison with other kinds of ducks. The muscles of A3 birds contained the most of UFA, however muscles of P33 birds contained the most of the SFA.

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The UFA/SFA ratio was more favourable for leg muscles (1.62-1.98) than for breast muscles (1.19-1.43), however for P33 muscles was the lowest. The UFA/SFA ratio for breast muscles was lower than for other kinds of ducks, and for leg muscles was alike. The PUFA/SFA ratio was on higher level (0.63-0.92) for all duck muscles than recommended (0.4). The PUFA n-6/n-3 ratio was 3.85-5.85 and was like recommended by the Nutrition Foundation (PUFA n-6/n-3 = 4-5) The lipids from A3 muscles were characterized by the best value of PUFA n-6/n-3 ratio (3.85.- breast, 4.57- leg muscles). This is very important, because the high level of the PUFA and especially the PUFA n-3 are preferred in human diets, as the most valuable in the UFA group, from the nutritional and physiological points of view. The lipids from A3 muscles were characterized by the best fatty acids profile among the investigated muscles.
Generally, basing on the results of our investigation we can say, that the muscles from the three Polish conservative duck flocks A3, K2, P33 were characterized by a low level of lipids and a high level of unsaturated fatty acids. It testified, that these muscles were favourable from the human nutrition point of view.

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Table: 1. Fatty acid composition of lipids from duck muscles

Fatty acid (%)	P3 3-B	P33-L	K2-B	K2-L	A3-B	A3-L
C_{16}	24.31^{a}	19.12^{b}	22.58^{a}	17.70^{b}	22.32^{a}	19.72^{b}
$C_{16:1}$	1.12	1.55	1.21	1.95	1.55	2.69
C_{18}	12.74^{A}	11.72^{bA}	13.78^{aB}	11.65^{bA}	10.69^{aC}	9.54^{bB}
$C_{18:1}$	22.07^{aA}	28.67^{bA}	22.31^{aA}	28.00^{bA}	25.07^{aB}	33.72^{bB}
$C_{18:1}$ (Σ of isomers)	2.76	3.93	3.02	3.65	3.71	3.36
$C_{18:2}$	14.28	13.97	14.68	14.43	12.52	13.46
$C_{18:3}$	1.62	1.11	0.83	1.12	1.00	1.22
C_{20}	0.19	0.13	0.16	0.08	0.13	0.05
$C_{20:1}$	0.41	0.43	0.31	0.40	0.41	0.44
$C_{20:2}$	0.37	0.23	0.33	0.23	0.25	0.16
C_{21}	0.60	0.48	0.38	0.42	0.85	0.58
C_{22}	0.79	0.80	0.60	0.36	1.23	0.46
$C_{20:4}$	7.03^{A}	7.65^{A}	10.17^{B}	10.15^{B}	8.56^{aC}	6.96^{bC}
$C_{20:5}$	0.59	0.28	0.44	0.27	1.09	0.43
$C_{22:4}$	0.80	0.75	1.01	0.98	0.82	0.68
$C_{22:6}$	1.97	2.73	2.98	2.95	3.38	2.82
Σ SFA	42.04	34.99	38.84	32.51	38.16	31.63
Σ MUFA	23.46	30.75	24.01	30.49	27.15	36.93
Σ PUFA	26.66	25.97	30.44	30.13	27.62	25.73
Σ UFA	50.12	56.72	54.45	60.62	54.77	62.66
Σ n-6	21.31	21.62	24.85	21.31	21.08	20.42
Σ n-3	4.18	4.12	4.25	4.18	5.45	4.47
UFA/ SFA	1.19	1.62	1.40	1.86	1.43	1.98
PUFA/SFA	0.63	0.74	0.78	0.92	0.72	0.81
n-6/n-3	5.09	5.23	5.85	5.66	3.85	4.57

The data are average value from 6 tests,
a,b - value with different letters at the same level differ within kind of muscle at $P < 0.05$,
A,B,C - value with different letters at the same level differ within kind of ducks at $P < 0.05$.

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EFFECT OF OIL SOURCES AND VITAMIN E LEVELS IN THE DIET ON THE CONCENTRATION OF TOTAL LIPIDS, CHOLESTEROL, VITAMIN E IN THIGH AND CHEST MEAT OF COCKERELS

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Keywords: cholesterol, lipids, vitamin E, cockerels

Abstract

Two hundred and forty 30-week-old White Leghorn cockerels were distributed in a completely randomized factorial arrangement of 5 X 2, using five oil sources (sunflower, soybean, canola, linseed and fish) and two levels of antioxidants (30 and 400 mg of vitamin E/kg of diet). The aim of the study was to evaluate the inclusion of different oil sources and vitamin E supplementation in the diet on the concentration of total lipids, cholesterol, vitamin E in cockerel meat. The intake of fish and canola oil in the diet reduced ($P<0.05$) the content of total lipids in the thigh meat as compared to the other oil sources. The highest content of total lipids was observed ($P<0.05$) in the breast meat of cockerels fed on linseed oil. A diet containing soybean oil also caused an increase ($P<0.05$) in the content of total lipids and cholesterol in the thigh meat as compared to the use of fish and canola oil. Vitamin E deposition was greater ($P<0.05$) in the thigh meat of cockerels fed on canola oil than on fish oil.

Résumé

Deux cents quarante coqs de la race blanche Leghorn, âgés de 30 semaines, ont été répartis au hasard en 5x2 lots correspondant à cinq sources d'huile (soja, cânola, tournesol, lin et poisson) et deux niveaux de vitamine E (30 et 400 mg/kg dans l'aliment). L´objectif de cette étude était d´evaluer l´inclusion de sources d´huile et vitamine E supplémentaires dans la diète et son effet dans la concentration totale de

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lipoïdes, colesterol et vitamine E dans la viande. Le fournissement d' huile de poisson et huile de cânola dans la nourriture a réduit (P<0.05) le contenu total de lipoïdes dans la viande de la cuisse comparé avec les autres sources de huile. Le plus grande contenu total de lipoïdes a été observé dans la viande de la poitrine des gallinacés qui ont été alimentés avec huile de lin. Une diète contenant huile de soja a causé aussi un accroissement (P<0.05) dans le total de lipoïdes et colesterol dans la viande de la cuisse comparé avec l´usage de la huile de poisson et de cânola. L´accumulation de vitamine E a été plus grande (P<0.05) dans la viande des cuisses des gallinacés alimentés avec cânola que avec huile de poisson.

Introduction

It is feasible to modify the lipid composition of poultry meat by dietary manipulation of fatty acids and to reach the nutritional guidelines, as for example the optimal ratios of PUFA/SAFA and ω6:ω3. Feedback inhibition of fatty acid synthesis in liver is greater during ingestion of unsaturated than saturated fat. Moreover, polyunsaturated fatty acids derived from dietary fat are more potent in the suppression of the activity of liver enzymes which participate in the synthesis of saturated and monounsaturated fatty acids (Sanz et al., 2000). In association with this recommendation, special emphasis has been given to the antioxidants. The aim of this study was to evaluate the dietary inclusion of different oil sources and levels of vitamin E, on the concentration of total lipids, cholesterol, vitamin E in cockerel meat.

Material and Methods

Two hundred and forty 30-week-old White Leghorn cockerels were housed in individual cages in a completely randomized factorial arrangement of 5 X 2, using five oil sources (sunflower, soybean, canola, linseed and fish) and two levels of antioxidant (30 and 400 mg of vitamin E/kg). The antioxidant used in this study was Lutavit E 50® (alpha-tocopherol acetate, BASF Corporation). Six percent oil was added regardless of the source and the total fat content of the experimental diets was 8% (isolipidic). The oils were given up by Bunge Foods S.A. The diet composition was based on the nutritional requirement according to NRC (1994). The principal fatty acids found in the diets can be observed in Table 1.
In the 54th week of age, the roosters were slaughtered, removing 4 birds/treatment, totaling 40 breast and thigh samples analyzed for cholesterol and total lipid contents. After separating the chests and thighs, the skins were removed, as well as all visible fat and connecting tissue. The lipids contained in the samples were extracted using the

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technique of Folch et al. (1957). In the poultry meat the results were expressed in mg/100g of edible portion, using the lipid conversion factor (F) reported by Holland et al. (1994). The cholesterol contents of the samples were determined after direct saponification according to Stewart et al (1992). Cholesterol was identified by comparison of the retention times of the samples with of the standard (Sigma C8667) and co-chromatography. Quantification was carried out by external standardization. The relationship between area and cholesterol concentration was linear in the range from 4.5 to 111.7 μg/ml, which covered the sample concentrations. The methodology used to determine the vitamin E content in the meet was that proposed by Brubacher et al. (1985). The data was assessed by analysis of variance using the System for Statistical and Genetic Analyses (UFV, 1997). Significant differences were determined by SNK test with a 5% level of probability.

Results and Discussion

In absolute values, the contents of total lipids and cholesterol were greater for thigh meat than for breast meat, which corroborates previous studies (Araújo, 1999). Probably the difference in the muscular composition, regarding the amount of lipids in the thigh and chest, is related to the different muscular functions and the need for giving skeletal support and movement. In the chest muscles of domestic birds, which are never used for flying, one can find a greater amount of type 2B muscular fiber, which have a minor oxidative metabolism, mioglobine and mitochondria, but have a bigger amount of glycogen.

The vitamin E level in the diet not affect the contents of total lipids and cholesterol in the thigh and chest meat ($P>0.05$). However, there was significant effect of oil source on these parameters. The content of total lipids deposited in thigh and chest meat of cockerels is illustrated in Figures 1 and 2, respectively. The offering of fish and canola oil in the diet reduced ($P<0.05$) the content of total lipids in thigh meat as compared to the other oil sources. In this way, there is a need to balance the ratio of dietary ω6:ω3 fatty acids, as was evident from the use canola and fish-oil based-diets which reduced the total lipids content in thigh samples. Thus when ω6:ω3 ratios of 6.62 and 8.38 were provided, by offering diets containing canola oil and fish oil, respectively, a desirable level was reached amongst the ω series, allowing the ω3 fatty acid to determine beta-oxidation and a reduction in hepatic lipogenesis. Previously, Phetteplace & Watkins (1990) reported that the effect of the ω3 fatty acids could be related to a greater beta-oxidation rate.

It was observed the highest content of total lipids ($P<0.05$) in the breast meat of cockerels fed the linseed-oil-based diet. The opposite was observed with previous studies (Ajuyah et al., 1991).

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The result of the cholesterol content in the thigh meat can be found in Figure 3. The use of soybean oil in the diet also showed an increase ($P<0.05$) in the content of total lipids (Figure 1) and cholesterol (Figure 3) in the thigh meat as compared to diets containing fish and canola oil. The influence of the soybean oil diet on the cholesterol content of the thigh meat is probably related to the level of saturated fat in the diet (Table 1), which was the highest amongst the oil sources of vegetable origin, being inferior only to the fish-oil-enriched diet. There was a significant effect ($P<0.05$) of the oil source on vitamin E deposition in the carcass. The data presented in Figure 4 show a higher amount of vitamin E in the thigh meat of cockerels fed on the canola-oil-based diet ($P<0.05$) than in that of cockerels fed on fish oil. The opposite was observed in the breast meat. The vitamin E deposition in the thigh was different from that in the breast, probably due to the type of fatty acid deposited in each type of meat.

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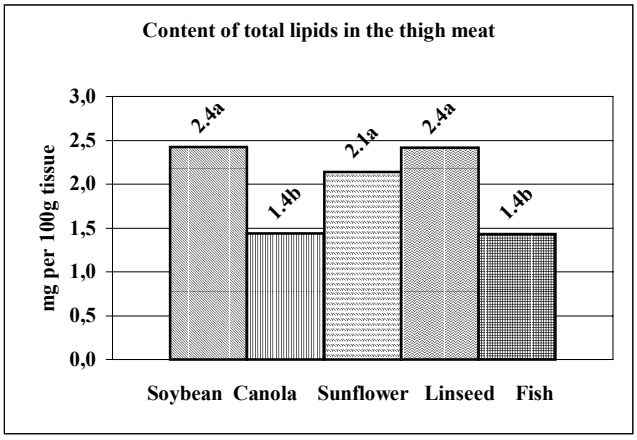
Table 1 - Profile of fatty acids found in the experimental diets

	Diets with oils of				
Fatty Acids (Area %)	Soybean	Sunflower	Canola	Fish	Linseed
Saturated	16.2	10.2	10.5	17.5	12.7
Monounsaturated	28.1	26.2	55.2	30.8	26.4
Polyunsaturated	55.7	63.6	34.3	51.7	60.9
omega-6 (ω6)	51.9	63.1	29.8	46.1	28.6
omega-3 (ω3)	3.8	0.5	4.5	5.5	32.3
Ratio of ω6:ω3	13.6	126.2	6.62	8.38	0.88
Unsaturated:Saturated (U:S)	5.17	8.80	8.52	4.71	6.87

Figure 1 - Content of total lipids in the thigh meat



[a,b] Averages values differ significantly if superscripts differ by the test Student Newman Keuls (P<0.05)

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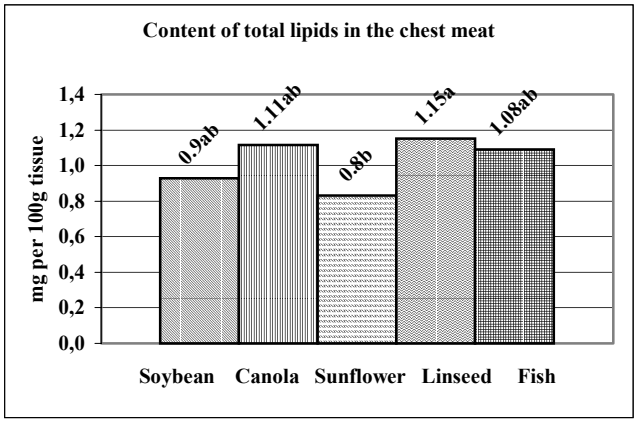
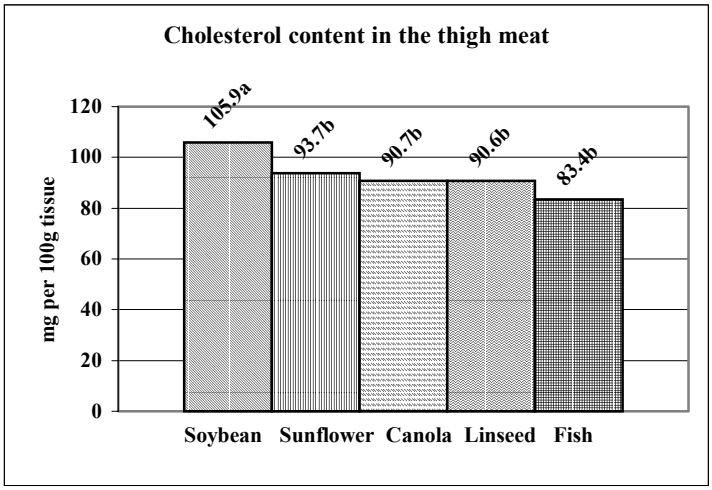
Figure 2 - Content of total lipids in the breast meat



[a,b] Averages values differ significantly if superscripts differ by the test Student Newman Keuls (P<0.05)

Figure 3 - Cholesterol content in the thigh meat



[a,b] Averages values differ significantly if superscripts differ by the test Student Newman Keuls (P<0.05)

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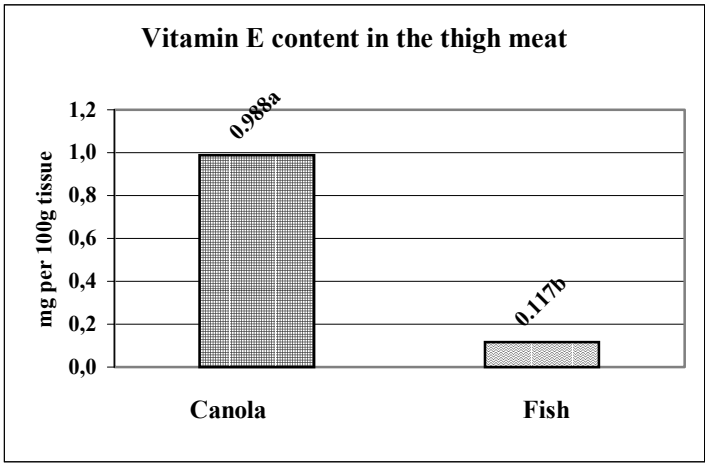
Figure 4 – Vitamin E content in the thigh meat



[a,b] Averages values differ significantly if superscripts differ by the F test (P<0.05)

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INFLUENCE OF LIPID (LINSEED OIL/LARD) AND VITAMIN E SUPPLEMENTATION IN FEEDING OF TURKEYS UPON THE OXIDATION OF PACKAGED COOKED-HAMS CURED WITH DIFFERENT LEVELS OF NITRITE.

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Keywords: linseed-oil, lard, vitamin E, cured-ham, oxidation

Abstract

The aim of this study was to examine the effect of dietary linseed oil, rich in n-3 PUFA's, compared to a saturated pork fat (lard), and vitamin E supplementation (30/400 ppm) on myoglobin and lipid oxidation of turkey cooked-ham cured with different levels of nitrite (50/100 ppm) and packaged in a modified atmosphere and in air. When animals are fed with 5% linseed oil and 400 ppm vitamin E, it is possible to use only 50 ppm sodium nitrite in curing to obtain a turkey cooked -ham of a good quality.

Résumé

Le but de ce travail sera d'examiner l'effet d'une supplémentation alimentaire des dindes en huile de lin, comparativement à du lard, et en vitamine E, sur l'oxydation des lipides et de la myoglobine de jambon cuit et nitrité (50 ou 100 ppm de nitrite) de dinde conservé sous atmosphère modifiée puis à l'air. Quand les animaux sont supplémentés avec 5% d'huile de lin et 400 ppm de vitamine E, il est possible d'utiliser seulement 50 ppm de nitrite de sodium dans la salaison pour obtenir un jambon cuit de dinde de bonne qualité.

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Introduction

Many meats, and particularly bovine meat, have been criticized on health because of high levels of saturated fatty acids presumed to increase the risk of heart disease in man. Conversely, polyunsaturated fatty acids (PUFA), which have particularly beneficial effects on health, are often present at low levels in meat , especially those of the n-3 series, and it is recommended to increase AGPI concentration of the n-3 serie compared to the n-6 one (Wood, 1999; Mossab, 2001). In this aim, it was decided to feed turkey with linseed oil because fatty acid composition of the poultry carcass is controlled by the type and amount of dietary fat. Nevertheless, this dietary regime richer in PUFA's can alter meat quality such as colour and flavour (Renerre, 2000) and conduct to a more fluid fat (Lessire, 2001). To slow oxidative processes, and as it was previously shown in the DIET-OX project (Mercier et al; 1998), antioxidants such as vitamin E, which is a "chain-breaking" component, must also be incorporated in the feeding of birds. The obtained results will be compared to those observed on turkeys fed a more saturated fat (lard). Moreover, in cooked cured-ham, nitrite is always used and is responsible of the pink colour of the product, the development of the well-loved flavor (by its antioxidative activity) and prevention of bacteriological deterioration such as the growth of *Clostridium Botulinum*. But nitrite can also react with amines and amino-acids in meat which produce carcinogenic N-nitrosamines (Cassens, 1995). To lower N-nitrosamines formation, a reduction of the level of nitrites in ham has been recommended with, in parallel, the use of antioxidants such as vitamin E. To-date, no alternative to nitrite has been identified although trials have been done for many years. The aim of this study was to examine the effect of a dietary linseed oil rich in n-3 PUFA's , compared to a saturated pork fat (lard), and of a dietary vitamin E supplementation, on myoglobin and lipid oxidation of turkey cooked ham cured with different levels of nitrite and packaged in a modified atmosphere for 4 weeks and in air for 6 days.

Material and methods

48 one day-old male turkeys (BUT 9) were used. They were allocated to a temperature and light-controlled floor pen with a wood shaving litter. Animals were fed pellets containing either linseed oil (5%) or saturated pork fat (5%) and vitamin E (α-tocopherol acetate) (30 ppm for control and 400 ppm for supplemented diets); water was provided at libitum.When they were 16 weeks old, animals were slaughtered in the slaughterhouse of the Poultry Research Station (Nouzilly). Muscle *Pectoralis major* was cut from the carcass, weighted and put under vacuum and sent in ice to the

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ADIV laboratory (Clermont-Ferrand) for the ham manufacturing. For the preparation of the cooked cured-ham, two different doses of sodium nitrite have been used: 50 and 100 ppm (100-150 ppm being the recommended dose in France). The brine was composed of nitrited salt (0,6%), sodium erythorbate, dextrose, lactose and polyphosphates. After fabrication, ham slices were stored in a modified atmosphere (70% N_2 / 30% CO_2) for 4 weeks; packages were then opened and ham slices were overwrapped in permeable PVC film and stored for a maximum of 6 days at 4°C in darkness. Results presented here have been obtained after the 6 days storage period. Colour stability was measured by spectrocolorimetry in the CIELAB system (1976) with calculation of luminosity (L*), redness (a*), yellowness (b*), hue (arc tg (b*/a*)and saturation $(a^{*2} + b^{*2})^{1/2}$(Renerre, 2000) in the meat colour laboratory. Metmyoglobin percentage was calculated by the Krzywicki method (1979) and discoloration index of nitrosopigment by the R570 / R650 ratio (Giddey, 1966). Lipid oxidation was measured by the TBA-RS content and the results were expressed as mg MDA / kg meat (Gatellier et al., 2001 (1)). The vitamin E content in meat was determined as described previously in the Roche Vitamines Laboratory (Gatellier et al., 2001(2)). Visual appreciation was also done in the meat colour laboratory by a five persons team and samples were noted (preference) on a five points scale. Mean values were compared by the Student "t" test.

Results

Turkeys fed linseed oil accumulated more C18:3 n-3 than turkeys fed lard and results will be presented in a next paper. The mean values for vitamin E concentration was 7.93 ± 0.55 ppm for hams made from vitamin E supplemented animals; for control animals, the mean values for vitamin E concentration was 1.30 ± 0.10 ppm. These results show that the vitamin E dietary supplementation was correct (x 6 / control) and identical to those previously obtained on fresh meat (Mercier et al., 1998). With these differences in vitamin E concentration, important differences in lipid oxidation measured by the TBA-RS test are noted between control and supplemented animals ($P < 5\%$) but with no difference in colour parameters (results not showed). These conclusions obtained on cured cooked-hams of turkeys are identical to those obtained previously on turkey meat (Mercier et al., 1998).

The results presented here have been obtained with animals supplemented with 400 ppm vitamin E. As previously recommended (Hunt et al., 1991), the colour changes of meat samples will be better appreciated by the a*/b* ratio than by the a* value alone. As shown in figure 1, and for a given oil (linseed oil (LO) / lard (l)), no significant difference in a*/b* was noted between 50 and 100 ppm nitrite. But, with linseed oil (LO50, LO100), a* /b*values are higher than those obtained with lard (l50, l100) (P <

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5%). These results are corroborated to those obtained with saturation (figure 2): saturation is greater (P< 5%) with animals fed lard and with meat nitrited with only 50 ppm nitrite (l50), instead of 100 ppm, corresponding to a greater oxidation rate (Gatellier et al., 2003). The metmyoglobin percentage (figure 3) shows that when animals are supplemented with linseed oil, the hams cured with 50 or 100 ppm sodium nitrite (LO50, LO100), give identical results but which are better than those obtained with the use of lard, particularly when 50 ppm of nitrite (l50) is used (Figure 3). Therefore, it is possible, even with a feeding supplementation of 5% linseed oil, particularly rich in PUFA's, but also with a 400 ppm vitamin E protection, to obtain a decreased oxidation of myoglobin at the surface of slices of cooked cured-hams stored first in modified atmosphere and, secondly, in air. For the discoloration index of nitrosopigment (R570/R650) (Gatellier et al., 2003), no difference was observed between samples (figure 4) whatever the lipid used in feeding of animals (linseed oil / lard) or the level of sodium nitrite (50/100 ppm). Pegg & Shahidi (1997) have previously observed that it was possible to obtain a pink colour in cooked ham if sodium nitrite was used at a minimum 50 ppm level. By visual appreciation (figure 5), it was observed that in birds fed linseed oil, the use of 50 ppm nitrite in the ham (LO50) was sufficient compared to 100 ppm (LO100). Conversely, with lard, the use of 50 ppm nitrite give bad results for visual appreciation (l50).

Whatever the dietary lipid in turkeys (linseed oil / lard), or the level of sodium nitrite in the cooked cured-ham (50/100 ppm), no difference was observed between samples in the TBA-RS test (figure 6). These experimental values confirm those previously obtained on the same samples after they were oxidized by chemical induction with the ascorbate / iron system (Gatellier et al., 2001(1)). The use of only 50 ppm of sodium nitrite is sufficient to lower lipid oxidation to a correct value which may lead to flavor deterioration. This is possible because, in this experiment, a high level of vitamin E was also used in feeding of animals and, consequently, a high level of vitamin E was found in meat, such as noted previously, beneficial from a nutritional and technological point of view. For Berthelsen et al. (2000), mecanisms which link liposoluble vitamin E to nitrite are always not elucidated. For Cassens (1995), and for unexplained reasons, in turkey meat, it was observed that residual nitrite level decreased in great quantity in transformed products. Actually, american recommandations are to use about 150 ppm nitrite near of the french ones (100-150 ppm) to be sure to prevent microbiological problems *(/ Clostridium botulinum*).

Previously, it was observed in the laboratory (Gatellier et al., 2001(1)), that after a chemical induction, with samples supplemented with only 50 ppm nitrite, but also with vitamin E, the protein oxidation rate (measured by the carbonyl content) was identical to the rate obtained with 100 ppm nitrite. Moreover, and with the use of linseed oil in animal feeding, results from sensorial analysis (to be presented) show no difference in flavour between samples cured with different levels of nitrite (50/100 ppm).

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Nevertheless, the residual quantity of nitrite found in the meat must be modulated by many factors such as the meat pH, the storage mode of the ham (residual oxygen %), and the storage temperature (Kilic et al., 2002). For Moller et al. (2000), the level of residual oxygen in the package must be equal to 0.1% maximum to avoid the nitroso-pigment discoloration in the presence of light. This maximum level of residual oxygen is identical to that found in bovine meat stored for long periods in 100 % CO_2 (Renerre & Labadie, 1993). For unexplained reasons, Kilic et al. (2002) have shown that after cooking, only 10 to 20% of nitrite initially added during ham curing was found after many weeks of storage.

Conclusion

When animals are supplemented with high quantities of vitamin E (400 ppm), it is possible to feed them with linseed oil at a 5% dietary level and, consequently, to improve the nutritional quality of meat and meat products. In these conditions, it is possible to use only 50 ppm nitrite (instead of 100-150 ppm usually recommended) to obtain turkey cooked cured-ham of a good quality from a colour and lipid oxidation point of view with less residual carcinogenic nitrosamines.

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Figure 1

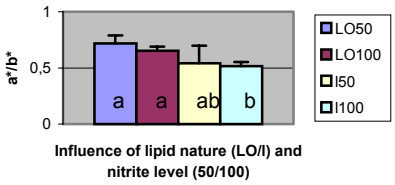


Figure2

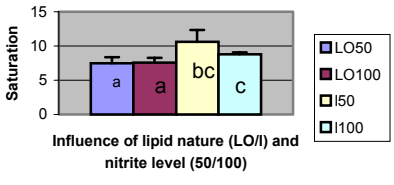
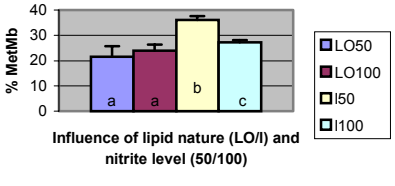


Figure 3



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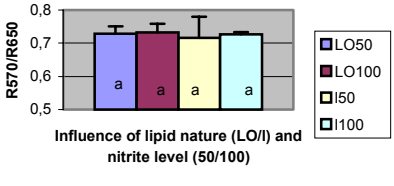


Figure 5

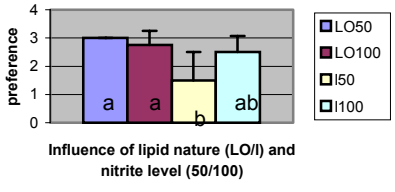
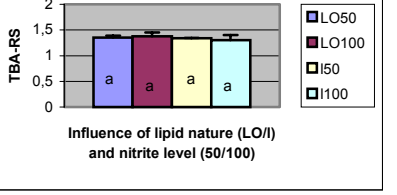


Figure 6



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TRANSFER OF α–TOCOPHEROL STEREOISOMERS FROM FEEDS TO POULTRY DEPOTS

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Keywords : α–Tocopherol Stereoisomers, Thigh, Liver, Poultry

Abstract

The present study was carried out to evaluate the transfer of different α-tocopherol stereoisomers from feeds to tissues (liver and thigh samples) in poultry. Fourty-eight female broiler chickens were distributed in 4 experimental treatments resulting from 4 levels of supplementation with α–tocopheryl acetate (0, 100, 200 and 400 mg/kg). The feeds supplemented with α–tocopheryl acetate contained similar proportion of each stereoisomer. The diets without α–tocopheryl acetate supplementation showed the following α–tocopherol stereoisomers: RRR 32.5%, RRS 25.6%, RSR 27.9%, RSS 12.8% and total 2S forms 1.1%. The stereoisomer profiles in liver and thigh were similar. Both tissues preferentially accumulated the 2R α–tocopherol forms (the 2R forms ranged from 69.2% to 93.4 %), with a predominance of the RRR and RRS forms. Consumption of α–tocopherol from dietary ingredients caused important differences in the stereoisomer profile in studied tissues as compared with those from animals fed the α–tocopherol commercial form, independently of α–tocopherol supplemented levels.

Résumé

Cette expérience a été mise en place pour évaluer le transfert de différents stéréo-isomères d'α-tocopherol des aliments au foie et à la cuisse des volailles. Quarante huit poulets de chair femelles ont été répartis dans 4 traitements expérimentaux correspondant à 4 niveaux de supplémentation en α-TA (0, 100, 200 et 400 mg/kg). Les aliments supplémentés en α-TA contenaient une proportion identique de chaque stéréo-isomère. Les régimes non supplémentés en α-TA contenaient les stéréo-isomères suivants de α-Toc : RRR 32.5%, RRS 25.6%, RSR 27.9%, RSS 12.8% et total 2S forme 1.1%. Les profils des stéréo-isomères dans le foie et la cuisse étaient

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équivalents. Les deux tissus accumulaient préférentiellement les formes 2R α-Toc (les 2R formes vont de 69.2% à 93.4%) avec une prédominance des formes RRR et RRS. La consommation de α-Toc en fonction de la composition des régimes entraînent des différences importantes dans le profil des stéréo-isomères dans les tissus étudiés, quand on les comparait avec ceux des animaux alimentés avec la forme commercial du α-Toc, indépendamment des niveaux supplémentés de α-Toc.

Introduction

All-*rac*-α–Tocopheryl acetate (α–TA) is the most common commercial vitamin E source used to supplement feeds. It consists of an equimolar ratio of all eight possible stereoisomers, which have distinctly different biological activities (Weiser and Vecchi, 1982). In general, 2R α–tocopherol (α–Toc) forms (RRR + RRS + RSR + RSS) have been shown to have higher biological activity than 2S α–Toc forms (SSS + SSR + SRS + SRR). Thus, 2R α–Toc forms are preferentially maintained in tissues of humans (Traber et al., 1990), rats (Nitta et al., 1993; Ueda et al., 1993; Kiyose et al., 1995; Weiser et al., 1996) and pigs (Lauridsen et al., 2002). However there is not information concerning transfer of α–Toc stereoisomers from feeds to tissues of chickens.

Material and Methods

Animals and diets

Fourty-eight female broiler chickens at one day of age were randomly distributed into 4 dietary treatments of three replicates each. The animals were housed in groups of four in twelve cages under standard conditions of temperature, humidity and ventilation. The diets were formulated according to exceed the requirements recommended by the NRC (1994) on the basis of 39% wheat, 34% soya and 13% barley (Table 1). The experimental treatments were resulting from 4 levels of supplementation with α-TA: without supplementation (E0) and supplemented (ES) with 100, 200 or 400 mg/kg. Feed and water were provided ad libitum during the 44 days of experimental period. Feed samples were taken three times during the experiment for α-Toc stereoisomer profile

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Sample collection

At the end of the experimental period, two animals per cage were killed in a commercial slaugterhouse. Boned thighs with skin and liver samples were freeze-dried, ground and stores at -20°C until further analyses.

α–Tocopherol steroisomer analysis

Ten µg of α–Toc were extracted from samples as described previously by Jensen et al (1999). Then α–Toc extracted was derivatized to their methyl ether following the method described by Drotleff and Ternes (2001). Chromatographic separation was performed according to the conditions described by Drotleff and Ternes (2001).

Table 1. Composition and chemical analysis of the basal diet[1].

Ingredients	**%**	**Chemical analysis**	**%**
Wheat	39.30	Dry matter	90.60
Soya 48	34.09	Crude protein	22.96
Barley	13.39	Crude fat	10,16
Tallow	9.00	Crude fibre	3.35
Bicalcium phosphate	2.17	Ash content	5.88
Calcium carbonate	0.98	Crude Energy (Kcal/kg)	4289
Salt	0.45	Metabolizable Energy (Kcal/kg)[3]	3100
Vitamin-mineral mix[2]	0.40		
DL-Methionine	0.28		
L-Lysine	0.04		

[1]Values are means of 4 dietary treatments.

[2] Vitamin and mineral mix per kg of feed: Vitamin A: 12000 UI; Vitamin D_3: 2400 UI; Vitamin K_3: 3 mg; Vitamin B_1: 2.2 mg; Vitamin B_2: 8 mg; Vitamin B_6: 5 mg; Vitamin B_{12}: 11 µg; Folic acid: 1.5 mg; Biotin: 150 µg; Calcium pantotenate: 25 mg; Nicotinic acid : 65 mg; Mn: 60 mg; Zn: 40 mg; I: 0.33 mg; Fe: 80 mg; Cu: 8 mg; Se: 0.15 mg.

[3] Estimated value.

Statistics

ANOVA was carried out to determine whether dietary α-Toc affected α-Toc stereoisomer content in liver and thigh of chickens. In all cases, $P \leq 0.05$ were considered significant.

Results and Discussion

Diet composition

α–Toc stereoisomer profile of experimental diets is shown in Table 2. As expected, feeds supplemented with α–TA contained similar proportion of each stereoisomer. On the other hand, diets without α–TA supplementation showed higher proportion of 2R forms (98.9%), mainly as RRR form (32.5%). The natural form present in the

ingredients of feeds is assumed to be only RRR form. However, this work shows that feeds non-supplemented with α–TA contain other stereoisomer forms different than RRR.

Liver and thigh composition

Consumption of different levels of α–TA did not lead significant differences in α–Toc stereoisomer profile in liver and thigh. Then, dates from treatments supplemented with α–TA were grouped and statistical analysis was carried out to compare natural (E0) and synthetic form (ES) of supplementation with α–TA, and they are shown in Table 3.

Table 2. α–Tocopherol stereoisomer profile of the experimental diets, expressed as %.

Stereoisomer	E0[1]	ES
RRR	32.5	14.1
RRS	25.6	15.2
RSR	27.9	11.6
RSS	12.8	11.4
Total 2R[2]	98.9	52.2
Total 2S[3]	1.1	47.8

[1] E0: without α–tocopheryl acetate supplementation; ES: supplemented with 100, 200 or 400 mg/kg α–tocopheryl acetate.
[2] Total 2R: RRR + RRS + RSR + RSS.
[3] Total 2S: SSS + SSR + SRS + SRR.

Stereoisomer profile in liver and thigh displayed a similar pattern. Those results corroborate findings of other authors that did not find differences in 2R stereoisomer profile of liver, brain, adipose tissue and other tissues of rats fed diets containing α–TA (Ueda et a., 1993; Weiser et al., 1996).

Table 3. Effect of α–tocopherol source on α–tocopherol stereoisomer profile in liver and thigh (expressed as %)[1].

Steroisomer	Liver				Thigh			
	E0[2]	ES	P	SEM	E0	ES	P	SEM
RRR	39.2	18.7	0.0001	0.833	53.1	19.4	0.0001	1.681
RRS	20.3	18.4	0.0768	0.523	21.0	23.3	0.4415	1.478
RSR	13.7	16.2	0.0257	0.537	0.0	14.9	0.0001	0.575
RSS	19.9	16.6	0.0038	0.527	19.2	11.7	0.0068	1.285
Total 2R[3]	93.1	69.9	0.0001	0.952	93.4	69.2	0.0001	1.284
Total 2S[4]	7.0	30.1	0.0001	0.950	6.6	30.8	0.0001	1.284

[1] Values given in this table correspondence to least-squares means obtained from ANOVA and their pooled SEM.
[2]E0: without α–tocopheryl acetate supplementation; ES: supplemented with 100, 200 or 400 mg/kg α–tocopheryl acetate.
[3] Total 2R: RRR + RRS + RSR + RSS.
[4] Total 2S: SSS + SSR + SRS + SRR.

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In general, both tissues accumulated predominantly 2R α–Toc stereoisomer. However 2R to 2S stereoisomer ratio (2R:2S) in studied tissues depended on dietary source of α–Toc. Thus, 2R:2S was 13.8 in E0 treatments and 2.3 in ES treatments. These results confirm those of other authors (Ingold et al., 1987) who showed that RRR to SRR ratio in brain of rats was relative to dietary ratio of these stereoisomers. Thus, consumption of natural α–Toc source with 99% of 2R forms resulted in a higher proportion 2R form in liver and thigh (93%). Whereas 2R forms in studied tissues from chickens fed α–TA were 70% in comparison to the 52% as present in diet. These results agree with findings of other authors who found 2R stereoisomer profile in liver of rats fed α–TA ranged from 71 to 85% (Nitta et al., 1993; Ueda et al., 1993; Kiyose et al., 1995).
Our results seem to confirm those from Weiser et al. (1996) who found a stereoisomer biodiscrimination towards 2R forms and confirmed that the configuration at C-2 of the α–Toc molecule has a major impact on stereoisomer biodiscrimination. Recent studies shown liver as critical in the biodiscrimination process of stereoisomers, as a result of α–Toc transfer protein (α–TTP). Nevertheless, α–TTP has not been reported in chicken, but in rats and humans (Sato et al., 1991, 1993; Kuhlemkamp et al., 1993). However, our results suggest the presence of α–TTP in chicken liver.

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ANTIOXIDATIVE PROPERTIES OF GRAPE SEED EXTRACT IN COOKED TURKEY MEAT

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Keywords : breast meat, refrigerated storage, oxidation

Abstract

Efficiency of grape seed extract to retard oxidative rancidity in cooked turkey meat was compared to Trolox C (α-tocopherol) and control (without antioxidants). Development in lipid oxidation during 13 days' refrigerated storage was evaluated by means of Thiobarbituric Acid Reactive Substances (TBARS) and hexanal formation. Treatment with antioxidants significantly improved oxidative stability of minced turkey meat during the storage period, although not to the same extent. The ability of grape seed extract to prevent lipid oxidation increased with increasing levels in the meat. Vacuum packaging improved oxidative stability of meat without or with low level of grape seed extract. It appears that grape seed extract could be very effective in inhibiting lipid oxidation of cooked turkey meat during chill-storage.

Résumé

L'efficacité d'extrait de pépins de raisin sur le rancissement de viande cuite de dinde a été comparée à Trolox C (α-tocophérol) et en comparaison contrôle (sans antioxydant). La stabilité oxydative des lipides pendant 13 jours en local réfrigéré a été évalué par Thiobarbituric Acid Reactive Substances (TBARS) et par la formation d'hexanal. Le traitement avec des antioxydants a significativement amélioré la stabilité oxydative de la viande de dinde hâchée pendant le stockage, mais pas toujours au même degré. La capacité d'extrait de pépins de raisin à empêcher l'oxydation des lipides est liée à son niveau dans la viande. L'emballage sous vide a amélioré la stabilité oxydative de la viande sans ou avec de bas niveau d'extrait de grains de raisin. Il paraît que l'extrait de pépins de raisin pourrait être très efficace pour empêcher l'oxydation des lipides de la viande cuite de dinde pendant le stockage en local réfrigéré.

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Introduction

One of the major problems occurring during processing, storage and final distribution of meat and meat products is lipid oxidation. This process initiates other changes, which adversely affects the product's colour, flavour, texture and nutritional value. The major strategies for preventing lipid oxidation are the use of free radical scavenger terminators such as phenolic antioxidants and restricting the access to oxygen during storage by vacuum-packing (Ahn et al. 2002; Mielnik et al., 2003; Shahidi et al., 1992).

In the past few years, various plant materials containing phenolic compounds have been proven in model systems to be effective antioxidants. Flavonoids, the most potent antioxidative compounds of plant phenolics have been found in vegetables, fruits, berries, herbs and tea leaves (Kandaswami and Middleton, 1997; Shahidi and Wanasundara, 1992; Skrede and Wrolstad, 2002).

Grape seed extract obtained as by-products of wine and grape juice processing is rich in proanthocyanidins i.e. long chain flavonoids. The multiple mechanisms of their antioxidative activity are expressed in ability of radical scavenging, metal chelation, and synergism with other antioxidants (Lu and Foo, 1999). Grape seed extract has been evaluated for its antioxidative effect in food. It has been reported to improve the oxidative stability of cooked beef (Ahn et al., 2002) and turkey thigh patties (Lau and King, 2003)

The purpose of the present study was to examine the efficiency of grape seed added on three different levels to retard lipid oxidation of cooked turkey meat stored under refrigeration.

Material and Methods

Four factors were studied in this experiment:

- Antioxidant type - the grape seed extract was compared with Trolox C a water soluble analogue of α-tocopherol (0.01%) and a control without antioxidant.
- Antioxidant concentration – grape seed were added to meat at three levels 0.04 %, 0.08% and 0.16 %.
- Storage conditions – samples were stored in aerobic or vacuum atmosphere.
- Storage time – the cooked meat samples were stored for 6 and 13 days at 4 °C.

Preparation of antioxidant solutions

Three concentrations of grape seed extract were defined on the basis of antiradical power (ARP), measured by means of the $DPPH^{\bullet}$ free radical scavenging method (Brand-Williams et al., 1995).

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Commercial grape seed pills were first grounded into a powder. All antioxidants were diluted in distilled water. A small amount of ethanol was added to help Trolox C and grape seed extract dilute in water.

Preparation and cooking of ground turkey meat

Turkey breast meat obtained from a commercial processing plant was minced in an industrial meat grinder in the pilot plant at the institute and later mixed with different antioxidants. Solution of 50 ml was added directly to meat using a laboratory blender. A control sample was formulated without antioxidant. All experimental batches were divided into smaller portions (100g) for sampling at each storage period and packaging conditions. The samples were vacuum-packed and cooked in a water bath at 80 °C for 30 minutes and cooled in tap water for 30 minutes. Then, half of the samples were pre-packaged into transparent boxes with air access while the other half was packaged in impermeable bags under vacuum. The meat samples were then stored at 4 °C for 6 or 13 days before analysis.

Analysis

The extent of lipid oxidation was monitored by Thiobarbitur Reactive Substances (TBARS) and hexanal formation as described earlier (Mielnik et. al., 2002). Two replications were carried out for all samples.

All data were subjected to analysis of variance (ANOVA). Tukey's test was used to determine significance ($P < 0.05$) of the mean values for multiple comparison.

Results and discussion

The antiradical power (ARP) of the grape seed extract was found to be almost one fourth (1,65 mg $DPPH^{\bullet}$/mg extract) than for Trolox C (5,88 mg $DPPH^{\bullet}$/mg), which explains the amounts applied in the experiment.

The effect of antioxidant treatments on TBARS values in ground cooked turkey meat during refrigerated storage in air or vacuum atmosphere is shown in Table 1. Significant differences between antioxidant treatments were revealed just after cooking. The control samples on the average had almost ten times higher TBARS values than the samples with Trolox C (0.01%). Grape seed extract also had an evident protective effect towards lipid oxidation occurring during cooking, though not to the same extent. Grape seed extract at level 0.16% retarded oxidation as effective as Trolox C and differed significant from samples with 0,08% and 0.04% supplementation.

Oxidative rancidity for all samples increased during storage, but the highest rises in TBARS over time were seen in the control samples without antioxidants and packaged in air (Table 1). Trolox C and grape seed extract at 0.16 % delayed the oxidation process in meat during the13 days' storage to a considerable extent. However, the

grape seed extract added at 0.04 % and 0.08 % were less efficient than Trolox C and at the end of storage period. TBARS values increased on average to 3.87 and 2.42 mg malonaldehyd/kg meat, respectively. The investigation showed that both antioxidant type and its concentration influenced oxidative stability of cooked turkey meat. This finding is in agreement with findings by Lai et al., 1991 and Mielnik et al., 2003 who reported a strong linear relation between the amount of an antioxidant (rosemary extract) supplemented to meat and TBARS values indicating the increase of efficacy with higher concentration.

Table 1. Effect of packaging atmosphere, antioxidant treatment and grape seed extract concentration on TBARS values in cooked ground turkey meat during storage.

Packaging atmosphere	Treatments	Storage time (days)		
		1	6	13
Air	Control	2.82 ax	6.21 ay	6.97 ay
	Trolox C 0.01 %	0.24 dx	0.36 ey	0.33 hy
	Grape seed 0.04 %	1.81 bx	4.96 by	5.74 bz
	Grape seed 0.08 %	0.71 cx	2.71 cy	3.65 cz
	Grape seed 0.16 %	0.36 dx	0.47 ey	0.68 gz
Vacuum	Control	2.82 ax	3.41 cy	2.97 dx
	Trolox C 0.01 %	0.24 dx	0.34 ex	0.35 hx
	Grape seed 0.04 %	1.81 bx	1.81 dx	2.01 ey
	Grape seed 0.08 %	0.71 cx	1.05 dey	1.19 fz
	Grape seed 0.16 %	0.36 dx	0.56 ey	0.57 gy

a-d Means with different letters within a column are significantly different at $p < 0.05$.
x-z Means with different letters within a row are significantly different at $p < 0.05$.

The TBARS values measured in meat stored in vacuum bags were lower compared to the samples stored under aerobic conditions. Removal of oxygen improved oxidative stability of meat especially those without antioxidant or with low level of grape seed extract.

Hexanal, the most abundant volatile compound, formed from omega 6-fatty acid was highly correlated with TBARS values ($r = 0.959$) as showed in Figure 1.

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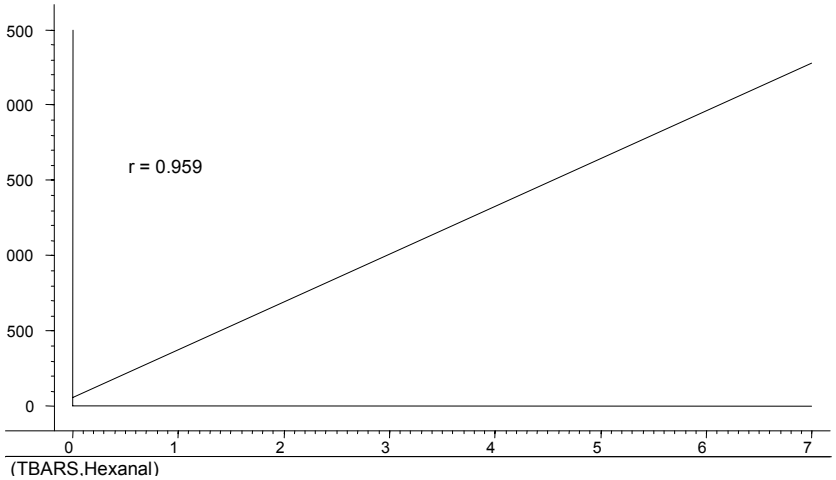

Figure 1. Correlation between TBARS values and hexanal measured in cooked turkey meat treated with antioxidants and stored at 4°C for 13 days.

Conclusions

Generally, supplementation of ground turkey meat with antioxidants before processing proved to be advantageous in regard of lipid stability of cooked meat chill-stored especially in air. The efficiency of grape seed increased with increasing antioxidant amount. The addition of grape seed extract together with vacuum packaging could be considered as a good way to improve lipid stability of cooked poultry meat.

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CONTENTS

IMPROVING THE NUTRITIVE VALUE OF BROILER MEAT BY FEEDING OPTIMUM VITAMIN NUTRITION (OVN™)

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Keywords: optimum, vitamin, nutrition, broiler, quality

Abstract

A trial was run with the aim to study the effect of using different vitamin levels in broiler diets (average level used in Spain vs. Optimum Vitamin Nutrition levels as recommended by Roche Vitamins) on animal performance, vitamin deposition in meat and meat quality, when broilers were subjected or not to density stress conditions similar to today's industrial broiler production conditions.
Performance parameters were measured at 20 and 40 days of age. At the end of the experiment, the effect of vitamin levels and stress on meat quality and breast meat yield was studied. Breast meat was also analyzed for vitamin content, drip losses and oxidative stability (TBARS values).
Feeding OVN™ improved performance and slaughterhouse yields as compared with feeding a control diet to broiler chickens from 0 to 40 days of age. High stocking density had a negative impact on most parameters studied. Breast meat of broilers fed OVN™ diets had significantly higher concentration of vitamins E, B1, and pantothenic acid, resulting in meat with a higher nutritional value than their control counterparts. Breast lipid oxidation was also reduced by the OVN™ premix. Therefore, the use of OVN™ premix improves broiler performance and yield, and enhances the nutritional quality of the broiler meat, with a clear benefit for the poultry industry.

Résumé

Cette expérimentation étudie les effets de deux niveaux vitaminiques dans l'aliment des poulets de chair sur la croissance, la dépôt des vitamines dans la viande et la qualité de la viande. Le niveau vitaminique témoin, reflet de la supplémentation

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moyenne réalisée en Espagne, est comparé au niveau OVN™ recommandé par Roche. Un facteur de stress, la densité (nombre d'animaux par m^2), a également été étudié afin d'être proche des conditions actuelles d'élevage.
Les paramètres zootechniques ont été mesurés à 20 et 40 jours d'age. A la fin de l'expérience, les effets du niveau vitaminique et du facteur de stress sur la qualité de la viande et le rendement filet ont été étudiés. Le contenu en vitamines, la perte d'eau et la résistance à l'oxydation (valeurs TBARS) ont également été analysés dans le filet de poulet.
L'aliment avec des niveaux élevés de vitamines (OVN™) améliore les performances zootechniques et le rendement à l'abattage des poulets de chair, par rapport à l'aliment contrôle. La densité la plus élevée a un effet négatif sur la plupart des paramètres étudiés. Comparé au groupe témoin, le filet des poulets supplémentés avec le niveau OVN™ présente des teneurs de vitamines significativement plus élevées en vitamine E, B1 et acide pantothénique, résultant en une viande de valeur nutritionnelle supérieure. De plus, l'oxydation lipidique est réduite dans le groupe OVN™. En conclusion, l'utilisation du niveau OVN™ améliore les performances zootechniques, le rendement en filet ainsi que la qualité nutritionnelle de la viande de volaille et apporte un net bénéfice à l'industrie avicole.

Introduction

Poultry vitamin levels in feed have been historically established by NRC (1994) and ARC (1984) to prevent clinical deficiencies and have changed only little over the last 30 years. During the same period of time, broiler feed conversion rates have improved drastically (more than 20%) due especially to a much higher body weight in a shorter production period. On the other hand, modern broiler production systems often place animals under high stress conditions so that an optimum level of vitamins in feed is essential to allow birds to achieve their full genetic potential and enhance health, welfare and therefore productivity
Poultry meat constitutes a large proportion of the meat consumed today, being an excellent source of essential protein as well as a good source of B-group vitamins. Besides, demand for further processed meat products have increased, as well as the interest of consumers for quality and nutritive meat. This involves continuous product development to create new value added products. Supporting these assumptions, recent consumer surveys (Hernandez and Seehawer, 2001) have shown that consumers consider the nutritive value of meat as one of the most important broiler meat quality factors.

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All these facts motivated some research to re-evaluate vitamin levels in feed in order to prevent an unbalanced animal nutrition and provide broiler meat to consumers with, at least, the same nutritional value than in the past.
Thus, the purpose of the present trial was to study the effect of using different vitamin levels in broiler diets on animal performance, vitamin deposition in meat and meat quality, when broilers were subjected or not to density stress conditions similar to today's industrial broiler production conditions.

Material and Methods

One thousand nine hundred and twenty one-day old male broiler chickens (Ross 308) were used. They were bulk weighed at arrival to the farm and distributed at random in 24 pens 5.5 m^2 each. There were four experimental treatments replicated six times and allocated randomly by blocks in the experimental rooms. The four treatments resulted from the factorial combination of two stocking densities and two feed vitamin levels. For treatments T-1 and T-2, there were 70 animals per pen, which represented a density of 12.7 animals/m^2. For treatments T-3 and T-4, as stressing factor, there were 90 animals per pen, which represented a density of 16.4 animals/m^2. Broilers from treatments T-1 and T-3 were fed a vitamin premix (Table 1) formulated according to the usual practice in Spain (Villamide and Fraga, 1999), whereas birds from treatments T-2 and T-4 were fed higher vitamin levels recommended by several worldwide specialists (Barroeta et al, 2002) and described in this paper as Optimum Vitamin Nutrition levels (OVN™).
Animals were weighed at the beginning of the experiment, at 20 days and at the end of the trial (40 days), and body weight gain, feed intake and feed to gain ratio was evaluated in each period and for the overall experiment. At the end of the experiment, five numerically identified chickens (using tags) from each pen were weighed and slaughtered. In order to evaluate the effect of vitamin levels and stress on meat quality, breast yield was studied. Both breasts of each identified chick were separated and weighed. Left breasts were used to determine drip losses (at day 0, 4 and 7 after 4º C storage) and one sample of the right breast was sent frozen to Roche Vitamins laboratory to determine vitamin content. Another aliquot of these right breasts was used to determine the TBARS (Thiobarbituric Acid Reactive Substances) values.
Data were analyzed according to the GLM procedure of SAS, to determine main effects of vitamin premix, stocking density, and the interaction between these factors. Storage time was used as a factor and its effect on TBARS values and drip losses was also determined.

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Results and Discussion

Treatments significantly affected all production parameters. Chickens fed OVN diets were heavier (P<0.01), ate more feed (P<0.01), and presented better average daily gain (P<0.04) than those fed control diets. Instead, high-density stress conditions statistically reduced average daily intake (P<0.001) and thus, lower growth rate of these animals was found (P<0.0001). Also, high stocking density negatively affected slaughterhouse results, and the birds reared under stress conditions had lower breast meat yield (P<0.01) than the controls. A summary of these results is shown in table 2; more detailed information on performance improvements was presented by the authors at the 11th European Poultry Conference in Bremen (Hernández et al, 2002).

Table 2. Performance parameters and breast meat yield

	BWT 40d g	**DWTG g/b/d**	**FI g/b/d**	**FCR g/g**	**Breast Yield % bw**
T1	2268	55.7	96.60	1.746	16.33
T2	2341	57.5	100.70	1.746	16.59
T3	2069	50.5	91.70	1.818	15.61
T4	2122	52.0	92.70	1.801	15.84
P					
(vit. level)	0.0433	0.0434	0.0111	ns	ns
(density)	0.0001	0.0001	0.0001	0.0026	0.0097
(vit. level *dens.)	ns	ns	ns	ns	ns

Higher levels (P<0.0006) of vitamin E, vitamin B1 in breast meat were achieved by feeding chicks the OVN premix (but not enough to be considered as vitamin enriched meat), thus improving the nutritive value compared to control broiler meat (Fig. 1,2). Feeding the OVN premix also increased breast meat panthotenic acid (P<0.0013); however, the effect was observed only in high stocking density conditions, thus leading to a premix*density interaction that was significant at P<0.0005.

Levels of the rest of vitamins were not modified by the OVN premix (results not shown). Stocking density had no effect on vitamin content in breast meat (results not shown).

Lipid oxidation (TBARS) of breasts was statistically affected by type of vitamin-premix (P<0.001), density stress conditions (P<0.021) and also storage (P<0.001). Breast meat of birds fed OVN premix showed lower TBARS values (0.27 *vs* 0.42 nmol/g) than animals fed control diets (Table 3). Breasts of chickens reared at high density presented slightly lower TBARS values, what could be related to their lower weight of abdominal fat and thus with their lower level of intramuscular fat.

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Drip losses obtained in breast of chickens fed enriched-vitamin diets were numerically lower than those from birds fed control feeds, but this difference failed to reach significance.

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Table 1. Composition of experimental vitamin premixes

Vitamins	T1, T3: Control (mg/kg)	T2, T4: OVN (mg/kg)
A: Retinol	13000 IU (26 g)	12500 IU (25 g)
D3: Cholecalciferol	2600 IU (5.2 g)	4000 IU (8 g)
E: alfa-Tocopherol	18.90	225.00
K3: Menadione	2.20	4.00
B1: Thiamin	1.40	3.00
B2: Rivoflavin	6.20	9.00
B6: Piridoxal	3.00	6.00
B12: Cobalamine	21.20	40.00
PP (B3): Niacin	33.00	60.00
B5: Pantothenic acid	10.40	15.00
M: Folic acid	0.68	2.00
H: Biotin	0.07	0.25
C: Ascorbic acid	0	100

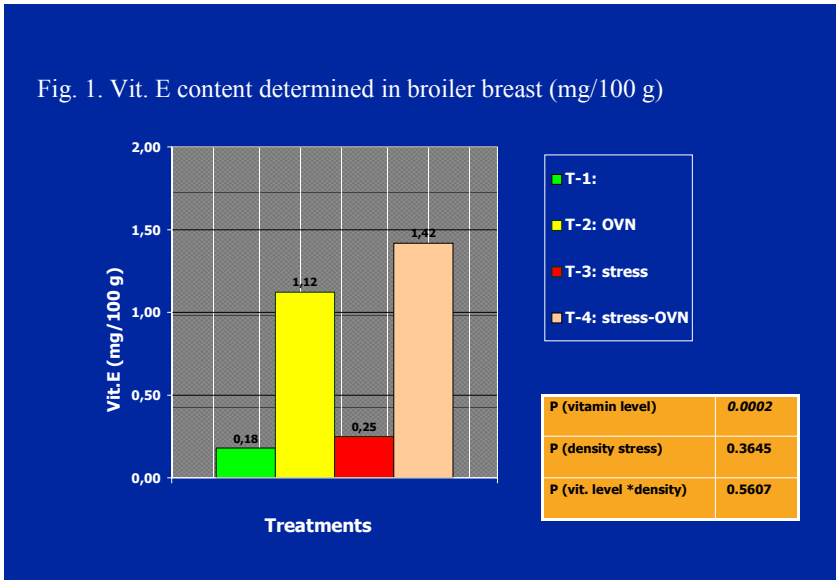

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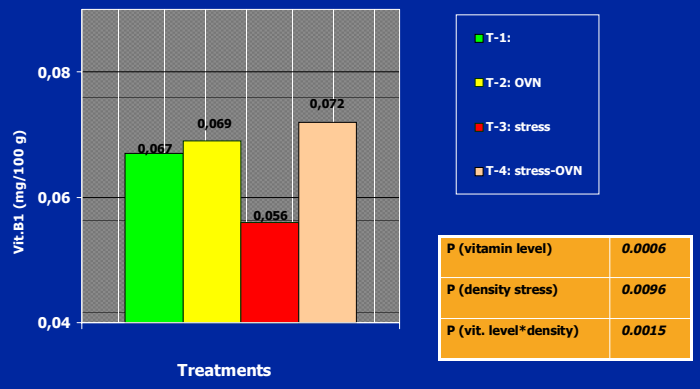
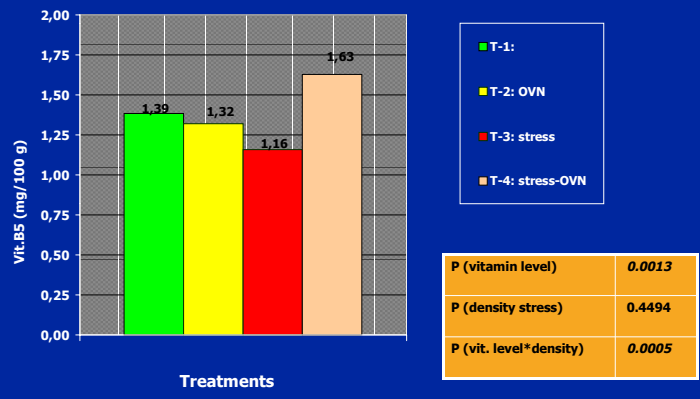
Fig. 2. Vit. B1 content determined in broiler breast (mg/100 g)



P (vitamin level)	*0.0006*
P (density stress)	*0.0096*
P (vit. level*density)	*0.0015*

Fig. 3. Pantothenic content determinded in broiler breast (mg/100 g)



P (vitamin level)	*0.0013*
P (density stress)	0.4494
P (vit. level*density)	*0.0005*

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Table 3. Drip losses and TBARS values determined in breast meat at slaughter

Treatment	Vitamin premix	Density (birds/pen)	Drip Losses (%)	TBARS values (nmol/g)
T-1	Control	70	6.79	0.51
T-2	OVN	70	5.76	0.28
T-3	Control	90	6.55	0.34
T-4	OVN	90	6.75	0.25
Vitamin premix effect:		Control	6.67	0.42 a
		OVN	6.25	0.27 b
		Pr>F	0.2819	**0.0001**
		Std.Err.	0.29	0.02
Density stress effect:		Without stress	6.25	0.39 a
		With stress	6.65	0.30 b
		Pr>F	0.2835	**0.0211**
		Std.Err.	0.29	0.02
Day effect:		0 days	4.28 b	0.27 b
		4 days	7.21 a	-
		7 days	7.86 a	0.42 a
		Pr>F	**0.0001**	**0.0001**
		Std.Err.	0.35	0.02

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BODY COMPOSITION AND MEAT SENSORY PROPERTIES OF BROILERS FED GREEN -OAK (*QUERCUS ILEX*), CORK OAK ACORNS (*QUERCUS SUBER L.*) BASED DIET.

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Keywords : oak acorns, broilers, meat

Abstract

Broilers chickens have been fed during six weeks diets containing (33 or 66%) of green oaks GO or oak cork acorns CO, compared to 66% of corn C. Lipids and proteins were determined in the thigh muscle. The meat tasting is achieved solely on chickens thigh of GO and CO 66% and corn diets. Feed based on oak cork acorn had generated a weak body weight, about 21% *vs* the corn diet. The same observation was showed for the abdominal fat proportion whose is reduced in chickens fed oaks acorns (1.46 against 2.8% of the carcass weight). Lipids in chicken thigh consuming the oak acorn diet exhibited a low content (4% against 6%). The difference of level in muscular protein are 17 to 24% than to the chicken of the control diet. A low tenderness and juiciness ($p < 0.01$) were noticed for the meat of chickens fed oak acorns: meat was rather dry. But, the flavor was judged globally low to high for the broilers meat fed oaks acorns compared to diet diet.

Résumé

Des poulets de chair sont nourris durant 6 semaines par des régimes contenant 33 ou 66% de glands de chêne vert (GO) ou de chêne liège (CO) et sont comparés à un régime témoin maïs. Les teneurs en lipides et protéines sont déterminées dans le muscle de la cuisse. Un profil sensoriel a été réalisé uniquement sur les cuisses de poulets des 2 régimes à 66% de glands et du régime standard. Les régimes à base de gland de chêne liège ont induit des poids vifs faibles (21% de réduction *vs* standard). Les mêmes observations sont valables pour le gras abdominal (1.46 contre 2.8% du poids de la carcasse). Les lipides de la cuisse des poulets consommant les glands de

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chêne sont moins élevés (4% contre 6% pour le standard). L'écart des teneurs protéiques musculaires est de 17 à 24% par rapport aux poulets du lot témoin. Une tendreté et une jutosité plus faibles ($p<0.01$) sont notées pour la viande issue des poulets de glands de chêne : la viande est plutôt sèche. Mais, d'une manière générale, la flaveur est jugée plutôt faible à élevée pour les poulets recevant les glands de chêne par rapport au régime standard.

Introduction

Oaks acorns, unexploited to date in Algeria, raise interest due to their large availability (27% of the forest surface), and their resistance to drought. The idea to exploit them for livestock feeding appeared in most Mediterranean countries as an energy source (47 to 60% of starch, Kekos and Kaukios 1985); 7% to 14.4% of lipids(Ofcaricks and Burns, 1971); some unsaturated fatty acid (UFA): oleic (66.8%), palmitic (18.4%), linoleic acids (13.5%) (Ofcaricks and Burns, 1971). During the past years, poultry growth increased but also their abdominal adipose tissue content. This fat is mainly lost during meat preparation. Feeding is one of the most important factors that can modify the quantity and the quality of lipids stored in chickens. In this fact, Bouderoua et al. (2002) have reported that comparatively to corn, diet those based on oaks acorns decreased the proportion of abdominal adipose tissue and linoleic acid proportion was higher than in chicken fed standard diet.
The present study was undertaken to study the effect of a diet based on green oak (*Quercus ilex*) or cork oak (*Quercus suber L.*) acorn in substitution to corn on body composition and sensory properties of meat of broilers chickens.

Material and Methods

500 males days- old-chickens (Indian River) were fed until 14 days with a standard diet, containing 3200 kacl/ Kg and 22% of protein. For each experimental diet 100 broilers were raised in floor pens (100chickens per pen). Initial body weight was 271 ± 26 g at 14 days of age. Five experimental diets based on oak acorns (green oak GO or cork oak acorn CO) and their incorporation rate (33 or 66%) in diet plus control feed (C) were given from 15 to 56 days of age (table I). At 56 days of age, chickens were weighed and slaughtered. Samples of thigh muscle were immediately frozen in liquid nitrogen and stored at –60°C until analysis. Lipids of muscular tissue were extracted by chloroform-methanol according to Folch et al. (1958) method. Proteins were analyzed using AFNOR method (1981). Meat sensorial properties were

appreciated solely on three diets broilers: GO 66, CO 66 and C. The total carcass was roasted and the test was concerned solely the high. Toughness , juciness and flavour were scored on a scale from to 1(low) to 10 (high) by a trained panel. All studied parameters were submitted to an analysis of variance including the effects of diets and incorporation rates (Dagnélie, 1980). Its followed by a using When applicable, Newmans and Keuls test was used for comparison of means.

Table I. Composition of experimental diets (%)

Diet	Corn	Green oak acorn	Green oak acorn	Cork oak acorn	Cork oak acorn
Ingredient	**C**	**GO 33**	**GO 66**	**CO 33**	**CO 66**
Corn	66	33		33	-
Green oak acorn	-	33	66	-	-
Cork oak acorn	-	-	-	33	66
Soybean meal	27	27	27	27	27
Wheat bran	5	5	5	5	5
MVC	1	1	1	1	1
Limestone	0.5	0.5	0.5	0.5	0.5
Phosphate	0.5	0.5	0.5	0.5	0.5
A M E (kcal/kg)	2928	2833	2868	2845	2839
Calculated composition (%)					
Crude protein	19.8	18.8	18.0	17.8	17.6
Lipids	3.3	4.2	4.0	3.8	3.7
Ash	1.3	1.0	2.1	2.0	2.8
Calcium	1.0	1.0	1.2	1.3	1.3
Phosphorus	0.5	0.4	0.4	0.3	0.4
Lysine	1.2	1.2	1.1	1.1	1.0
Sulfur amino acids	0.9	0.8	0.7	0.7	0.6

MVC: mineral vitamin premix, provided (in mg per kg of diet); vitamin E: 6; vitamin K_3: 0.80; vitamin B_1: 1; vitamin B_2: 3; Pantothenate de Ca: 6; vitamin B_6: 1.5; vitamin B_{12}: 0.006; folic acid: 0.2; nicotinic acid: 12; copper: 5; cobalt: 0.65; manganese: 65; zinc: 65; selenium :0. 25, iron.: 50; iodine: 0.8; magnesium: 100.

Results and discussion

Diets based on oaks acorns reduced significantly chickens growth (table II), especially with CO66 ($p<0.01$), about 21% compared to the control diet.

The same tendency was globally observed (table II) with abdominal adipose tissue proportion (1.46 to 1.68% of carcass weight in acorns diets *vs* 2.8% in C diet).

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The level of total lipids (table III) in thigh muscle tissue was significantly higher in C fed chickens than in those fed oaks acorns, especially CO66 (3.7 *vs* 6.1g/100 g of muscle tissue). Proteins levels were also lower ($p<0.01$) in broilers fed oaks acorns than in diet control C (17.1 *vs* 22.6g/100g of muscle). However, no significant effect of the diet and incorporation rate on dry matter could be measured (table III).

Table II: Mean body weight and abdominal adipose tissue (AAT) characteristics of broilers fed acorn based and control diets

Diet	Corn	Green oak acorn	Green oak acorn	Cork oak acorn	Cork oak acorn	SEM	Effect		
	C	**GO 33**	**GO 66**	**CO 33**	**CO 66**		D	SR	D.SR
Body weight (g)	2970[a]	2730[b]	2620[c]	2450[d]	2343[e]	9.86	**	*	*
Weight gain *(g)*	2708[a]	2468[b]	2358[c]	2188[d]	2081[d]	9.84	*	*	*
Eviscerated weight *(g)*	2108[a]	1965[b]	1886[c]	1727[d]	1701[d]	8.28	**	*	*
Abdominal adipose tissue *(g)*	59[a]	33[b]	29[b]	25[c]	25[c]	1.67	**	NS	*
(% of eviscerated weight)	2.8[a]	1.68[b]	1.55[c]	1.49[d]	1.46[d]	0.24	**	*	*

Each value is the mean of 30 chickens (body characteristics)
[1] ANOVA *P < 0.05 ; * * p < 0.01 ; NS : non significant.
Means values within a line having different superscripts are significantly different
D. diet , SR. substitution rate,

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Tableau III: Composition thigh muscle of chickens (g/100g fresh muscle)

Diets	Corn **C**	Green oak acorn **GO 33**	Green oak acorn **GO 66**	Cork oak acorn **CO 33**	Cork oak acorn **CO 66**	SEM	Effect D	SR	D.SR
Dry matter	25.8[a]	25.4[a]	25.8[a]	25.6[a]	25.6 [a]	1.16	NS	NS	NS
Protein	22.6[a]	20.5[b]	18.7[c]	20.3[b]	17.1[d]	1.05	**	*	*
Total Lipids	6.1[a]	4.8[b]	3.9[c]	4.7[b]	3.7[c]	0.72	**	*	*

Each value is the mean of 08 chickens
ANOVA *P < 0.05 ; * * p < 0.01 ; NS : non significant.
Means values within a line having different superscripts are significantly different
D. Diet, SR. substitution rate,

Comparatively to control diet C, meat of broilers fed oaks acorns was more tough ($p<0.01$) (Table IV). The same observations were valid in meat juiciness, in which the oaks acorns diets had involved a dry meat (5.75 *vs* 6.1 in corn diet C). Reciprocally, the meat broiler fed oak acorns was judged ad rather as lower in flavor ($p<0.01$).

Table IV: Results of tasting panels

Diets	Corn **C**	Green oak acorn **GO 66**	Cork oak acorn **CO 66**	SEM	Diet effect
Toughness (1)	5.61[a]	6.05[b]	7.22 [c]	1.04	*
Juiciness (2)	6.11 [a]	5.75 [b]	6.40 [a]	1.12	*
Flavor (3)	8.21[a]	7.22 [b]	5.83 [c]	1.05	**

Each value is the mean of 08 chickens
ANOVA *P < 0.05 ; * * p < 0.01 ; NS : non significant.
Means values within a line having different superscripts are not significantly different
(1) thougness was scored 1 to 10 whose : 1 to 5.9 is tender and 6 to 10 is though
(2) juiciness was scored 1 to 10 whose 1 to 5.9 is dry and 6 to 10 is juicy
(3) flavor was scored 1 to 10 whose 1 to 5..9 is judged as lower in flavor and 6 to 10 is higher

The low body and gain weight obtained with acorn-based diets, especially CO66, were attributed to a low protein concentration and may be availability of amino acids in the diets(especially sulfur amino acids). Indeed, the maximal chickens growth is reached at 20% of protein (Grisoni et al, 1990). The protein concentration of acorns is low (Short, 1976; Brigs and Smith, 1989), compared to corn (Leclercq et al., 1984). It is also possible that tannin, a major anti nutritional factor in acorns (Short, 1976), make amino acids less available for chickens.
A low digestibility of energy in Go and Co diets could explain the low fat deposition, similar to that of chickens genetically lean (Legrand and Lemarchal, 1987).

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Reciprocally, body weight and abdominal fat deposition observed in C diet are similar to those mentioned in literature.
The low level of muscular protein in chickens fed acorns diets could be explained probably by the low utilization of dietary protein, which in was complied by tannins acorns [Martin- Tangy, 1977; Marquardt et al., 1977)
Globally total lipids levels in muscle were lower in chickens fed oak acorns, than those mentioned: 5.1 g/100g of muscle. Indeed, the lipid of chicken's meat was affected by protein/energy ration of diet (Marion and Wood roof 1966, Marion et al. 1967).
The chickens fed on oaks acorns, have generated the low juicy of meat The consequences were probably attributed to low intra muscular collagen solubility, affected by tannins of acorns (Nakamura et al.,1975).
Finally, oaks acorns diets decreased growth performance, but also abdominal fat proportion in the carcass. However, lipid content of meat was lower. This observation requires further research on the nutritive value as on fatty acids in meat of chickens fed acorns.

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EFFECT OF VIRGINIAMYCIN IN DIETS ON PERFORMANCE AND DRESSING YIELDS OF BROILER

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Keywords: broilers, virginiamycin, dressing yields

Abstract

Commercial broilers were fed from 0 to 6 weeks of age with diets containing 0, 5, 10, 15 and 20 mg/kg of Virginiamycin. The growth rate and feed efficiency were significantly ($P < 0.01$) improved due to Virginiamycin supplementation. The mortality and feed consumption were not affected. The small intestine weight and length were reduced significantly ($P < 0.05$) with proportionate increase in the ready-to-cook yield, in all the Virginiamycin supplemented treatments. The giblet yield reduction due to virginiamycin supplementation was not significant.

Résumé

Des poulets de chair ont été nourris de 0 à 6 semaines avec des aliments contenant 0, 5, 10, 15 et 20 mg/kg de Virginiamycine. La croissance et l'efficacité alimentaire ont été significativement ($P < 0.01$) améliorées par la supplémentation en Virginiamycine. La mortalité et la consommation alimentaire n'ont pas été influencées par cette supplémentation. Le poids et la longueur de l'intestin grêle ont été significativement diminués ($P < 0.05$) avec une augmentation proportionnelle du rendement en carcasse « prête à cuire » dans tous les lots supplémentés en Virginiamycine. La diminution du pourcentage des abats due à la supplémentation en Virginiamycine n'était pas significative.

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Introduction

Virginiamycin is the most commonly used antibiotic in the broiler feed. Apart from bodyweight and feed efficiency in broilers, the processing yields, especially the breast yield has to be improved. Hence a study was undertaken to find out the ideal level of virginiamycin in broiler feeds for optimal economy, to study the influence of virginiamycin on the biometry of the small intestine and carcass yields.

Material and methods

Three hundred, straight run, commercial broiler chicks were randomly divided into 15 groups of 20 chicks each. Three replicates were randomly allotted to each of the five dietary treatments consisting of 0, 5, 10, 15 and 20mg of virginiamycin per kg feed. Except for the levels of virginiamycin, all feeds and management were common. Besides the data on body weight, feed consumption, mortality, the relative economy of raising broilers up to six week was calculated. At the end of sixth week, six male and six female broilers from each treatment were processed. The length and weight of the small intestine were measured. The weights of the eviscerated carcass, giblets and the ready-to-cook yields were recorded. All the data obtained in the study were subjected to analysis of variance for statistical significance under completely randomised design, according to the methods of Snedecor and Cochran (1989).

Results and Discussion

Virginiamycin supplementation significantly ($P<0.01$) improved the growth rate and thereby the feed conversion efficiency ($P<0.05$) without any significant change in feed consumption and mortality. Similar conclusions were drawn by Miles *et al.* (1984), Chotinski *et al.* (1985) Kantharaj (1990) and Kumararaj *et al.* (2000). On the other hand, Boorman (1984) and Henry *et al.* (1987) observed no significant differences which might be due to better quality of the basal feed used by them or low microbial load in the flock which may not need an antibiotic growth promoter.

In spite of additional cost of inclusion of virginiamycin in feed, due to better weight gain and F.C.R., the cost of production per unit broiler was 2.1 to 9.1% lesser in virginiamycin groups than the control. The group supplemented with 10 ppm of virginiamycin gave the least cost broiler production. Similar conclusions were drawn by Simon *et al.* (1987) and Kumararaj *et al.* (2000).

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The small intestine length and weight were reduced due to virginiamycin, leading to better absorption to nutrients and lesser dressing loss. Lighter intestines and giblets in virginiamycin groups resulted in higher dressing yields compared to control (+ 0.7 to 2.0%), which will earn more profits to the processors. Leeson (1984), Woodard *et al.* (1988) and Kumararaj *et al*. (2000) also reported increased carcass yields due to virginiamycin.

Performance of broiler as influenced by dietary virginiamycin levels

Trait	***Dietary virginiamycin levels (mg/kg)***				
	0 (control)	***5***	***10***	***15***	***20***
Daily weight gain** (g)	43.0^{a}±0.09	45.1^{ab}±0.94	45.6^{ab}±0.98	45.9^{ab}±0.84	47.8^{b}±1.04
Feed consumption/ bird (g)^{NS}	3486±28.6	3618±104	3524±33.6	3566±48.6	3674±25.9
Feed conversion ration*	1.93^{a}±0.03	1.91^{ab}±0.03	31.84^{b}±0.02	1.85^{b}±0.02	1.83^{b}±0.02
% Mortality	1.67	1.67	--	3.33	1.67
Relative cost of production (%)*	100^{a}	97.9^{b}	90.9^{c}	91.2^{c}	91.1^{c}
Small intestine weight (g/100g body weight)*	4.00^{b}±0.10	3.80^{ab}±0.06	3.64^{a}±0.05	3.70^{a}±0.01	3.76^{a}±0.02
Dressing yield (g/100g body weight)^{NS}	70.2±0.25	71.1±0.44	70.9±0.55	71.7±6.7	72.2±0.45
Giblets yield (g/100g body weight)^{NS}	5.19±0.22	4.79±0.24	5.16±0.19	4.90±0.14	4.86±0.06

* Means within each category bearing at least one common superscript do not differ significantly (P>0.05)

NS: Not significant

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CONTENTS

INFLUENCE OF DIETARY PEARL MILLET, PELLETING AND ENZYME SUPPLEMENTATION ON BROILER PERFORMANCE AND DRESSING YIELDS

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Keywords: Pearl millet, enzymes, pelleting, broilers, dressing yields

Abstract

In a 3 x 2 x 2 factorial experiment, pearl millet (*pennisetum typhoides*) was incorporated in broiler diets at 0, 250 and 500 g/kg, replacing corn by 0, 50 and 100 percent on w/w basis. All these 3 diets were offered to broilers, either as mash or pellets, with or without multi-enzyme cocktail supplementation. Significantly ($P< 0.05$) better growth rate and feed efficiency were noticed with pearl millet and pelleting. Enzyme supplementation was not beneficial. The ready-to-cook yield, gizzard and liver weights were not affected due to the dietary cereals as well as enzyme supplementation. On the other hand, pelleting reduced the gizzard size significantly ($P < 0.05$) but did not alter the carcass yield neither liver weight significantly. Hence, 100% replacement of corn with pearl millet along with pelleting will be beneficial to the broiler farmers.

Résumé

Dans un plan factoriel 3 x 2 x 2, des graines de millet (*pennisetum typhoides*) ont été incorporées dans des aliments pour poulets de chair à 0, 250 et 500 g/kg, en remplacement du maïs à 0, 50 et 100 % sur la base m/m. Ces 3 aliments ont été distribués aux poulets sous forme de pâtée ou de granulés, avec ou sans une supplémentation en enzymes. La croissance et l'efficacité alimentaire ont été significativement améliorées avec l'utilisation des graines de millet et la granulation. La supplémentation en enzymes n'a eu aucun effet. Le poids de la carcasse « prête à cuire », du gésier et du foie n'ont pas été influencés par la céréale, ni par la supplémentation en enzymes. Par contre, la granulation a réduit significativement ($P <$

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0.05) la taille du gésier sans altérer le rendement carcasse ni le poids du foie. Le remplacement du maïs à 100 % avec les graines de millet et la granulation sont des solutions intéressantes pour les éleveurs de poulets.

Introduction

Pearl millet (*Pennisetum typhoides*) is having 32% more protein, two times more lysine, 39% more methionine, four times more calcium and 40% more phosphorus with 15% lesser ME than corn (Narahari, 1997). If this partial deficit in ME is met by supplementing a multi-enzyme premix; pearl millet will be a better substitute for corn. Moreover, pearl millet is having lesser incidence of mycotoxins and higher levels of cardiac friendly ∝-linolenic acid and relatively cheaper than corn. This experiment was conducted to study the nutritional value of pearl millet as a total substitute for corn in broiler diets; with and without multi-enzyme supplementation.

Materials and methods

A 3 x 2 x 2 factorial biological trial was conducted to study the influence of pelleting and enzyme supplementation on the nutritional value of pearl millet (*Pennisetum typhoides*) for broilers. A total of 648 Ross broiler chicks was randomly divided into 36 groups of 18 chicks each. These replicates were randomly allotted to each of the 12 dietary treatments comprising three levels of millet replacing corn with or without pelleting and with or without enzyme supplementation. Irrespective of the treatment, all the chicks were fed *ad libitum* with the respective broilers starter and finisher feeds, from 0-3 and 4-6 weeks, respectively. Pearl millet was incorporated replacing corn at 0, 50 and 100 per cent levels in both starter and finisher diets on w/w basis. The above feed was subjected to pelleting and multi-enzyme supplementation at 500 g/tonne in both broiler starter and finisher feeds, throughout the experimental period. The data on body weight, feed consumption, mortality and their causes were recorded. The feed efficiency was worked out. The Broiler Farm Economic Index (BFEI) was calculated by using the formula proposed by Narahari (1996).

$$\text{BFEI} = \frac{\text{Live weight in kg x per cent livability}}{\text{Feed efficiency x days to reach market weight}}$$

At the end of six weeks three male and three female broilers from each treatment were randomly picked up, weighed and subjected to detailed slaughter studies. The data on various dressing yields and losses were measured. The abdominal fat pad thickness,

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shank length, keel length and breast angle were measured. All the data generated in this study were subjected to analysis of variance for significance according to the methods of Snedecor and Cochran (1989).

Results and discussion

The results shown in the Table revealed that the broiler fed pearl millet had performed better than corn in many aspects; especially the growth rate. Higher protein, lysine and methionine in pearl millet than in corn might be responsible for this additional growth rate. Reddy *et al.* (1987), Narahari and Rajini (1999) and Kumararaj *et al.* (2000) also reported superior performance of birds, when corn was replaced with pearl millet. Irrespective of the grains and enzyme supplementation, pelleting had resulted in better performance than mash; which concurs with the earlier findings of Rajini *et al.* (1998) and Kumararaj *et al.* (2000). Multi-enzyme supplementation had not improved the feed quality and broiler performance.

A combination of 100% millet with pelleting gave the heaviest broilers; while 100% corn pelleted diet gave best feed efficiency, due to higher energy and lesser wastage.

The mortality rate, ready-to-cook meat yield, keel length and fat pad thickness did not show any variations between treatments, indicating that pearl millet is a good substitute for corn.

The gizzards and giblets were smaller in pellet fed broilers than mash; irrespective of the grains and enzyme. Rajini *et al.* (1998) and Kumararaj *et al.* (2000) also noticed smaller gizzard in pellets fed broilers.

The breast angle was directly proportional to the body weight gain; indicating that in heavier birds, the breasts are wider. The Broiler Farm Economy Index (BFEI) was infavour of millet and pellet fed broilers; suggesting that where ever pearl millet was available, it can replace maize and pelleting will further improve its quality.

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Influence of dietary pearl millet, multi-enzyme supplementation and pelleting on the performance of broilers

Treatment	*Daily body weight gain** (g)*	*Feed efficiency**	*% mortality NS*	*% giblet weight**	*% ready-to-cook weight NS*	*Keel length NS*	*Breast angle**	*Fat pad thickness mm NS*	*Broiler farm Economy Index**
0% millet, no enzyme, mash	42.9^{a}±1.27	1.96^{ab}	7.41	6.4^{a}	71.7	11.2	62^{a}	51	1.90^{a}
0% millet, no enzyme, pellet	45.1^{ab}±0.97	1.81^{b}	7.41	5.4^{b}	72.3	11.2	69^{b}	53	2.02^{bcd}
0% millet, enzyme mash	43.0^{a}±1.03	1.92^{ab}	9.26	6.4^{a}	71.3	10.8	62^{a}	54	1.93^{ab}
0% millet, enzyme, pellet	44.9^{ab}±1.18	1.84^{b}	7.41	5.5^{b}	70.9	10.9	67^{ab}	55	2.01^{bcd}
50% millet, no enzyme, mash	45.0^{ab}±1.35	1.99^{ab}	9.26	6.4^{a}	70.7	11.3	64^{ab}	50	1.95^{abc}
50% millet, no enzyme, pellet	46.5^{b}±1.07	1.91^{b}	5.56	5.6^{b}	71.1	11.5	72^{bc}	51	2.04^{cd}
50% millet, enzyme, mash	44.6^{ab}±1.31	2.00^{a}	11.12	6.5^{a}	70.9	11.1	72^{bc}	51	1.96^{bc}
50% millet, enzyme, pellet	46.6^{b}±1.33	1.93^{ab}	9.26	5.6^{b}	71.7	11.1	74^{bc}	52	2.00^{bcd}
100% millet, no enzyme, mash	48.2^{bc}±1.58	2.07^{a}	7.41	6.3^{a}	71.3	11.3	74^{bc}	50	2.00^{bcd}
100% millet, no enzyme, pellet	49.6^{c}±1.31	1.94^{ab}	9.26	5.5^{b}	71.8	11.1	73^{bc}	52	2.01^{bcd}
100% millet, enzyme, mash	47.5^{bc}±1.27	2.05^{a}	5.56	6.4^{a}	70.3	11.5	77^{c}	51	2.01^{bcd}
100% millet, enzyme, pellet	48.8^{bc}±1.15	1.98^{ab}	9.26	5.7^{b}	71.2	11.5	76^{c}	53	2.06^{d}

Means with in each column bearing at least one common superscript do not differ significantly.
** Highly significant, * Significant, NS: Not significant

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THE EFFECTS OF DIETARY PROTEIN AND LYSINE LEVELS ON BROILER PERFORMANCE, CARCASS CHARACTERISTICS, AND NITROGEN EXCRETION

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Keywords: lysine, protein, broiler, performance, carcass characteristics

Abstract

The effects of dietary protein and lysine levels on performance, and carcass characteristics of Ross male broiler chickens from 1 to 3 weeks and 4 to 6 weeks of age were tested. Dietary treatments consisted of three levels of L-lysine.HCl in starter and grower period (0.0, 1.5, and 3.0 g/kg) and two levels of protein (208.4, 178.4 in starter, and 181.2, 161.2 g/kg in grower period) with 2900 kcal AME/kg diet.
Reducing dietary protein decreased weight gain in starter, grower, and total period up to 6.0, 4.6, and 5.6 % respectively ($P<0.05$). It also decreased feed consumption in starter period, and nitrogen excretion. Decreasing dietary protein had no significant effect on gain to feed ratio, and breast meat yield, but increased abdominal fat percentage significantly ($P<0.05$). Increasing dietary lysine increased feed consumption in starter and weight gain, and feed to gain ratio in grower and total period of the experiment ($P<0.05$). It also increased breast meat yield. Dietary treatments had no significant effect on mortality.

Résumé

Les effets des teneurs en protéines et en lysine de l'aliment sur les performances de croissance et sur les caractéristiques des carcasses de poulets mâles Ross ont été testés entre 1 et 3 semaines et 4 et 6 semaines. Les régimes alimentaires comportaient 3 niveaux de L-lysine HCl pour les périodes de démarrage et de croissance (0.0, 1.5 et 3.0 g/kg) et deux niveaux protéiques (208.4 et 178.4 pour la période de démarrage et 181.2 et 161.2 g/kg pour la période de croissance) avec 2900 kcal EM/kg d'aliment. La

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réduction du taux protéique alimentaire s'est traduite par une diminution du gain de poids sur les périodes de démarrage, croissance et sur l'ensemble de la période d'élevage de 6.0, 4.6 et 5.6 % respectivement. Elle a induit également une diminution de la consommation alimentaire pour la période de démarrage et de l'excrétion azotée. Par contre, elle n'a eu aucun effet sur l'indice de consommation ni sur le rendement en filet, mais elle a entraîné une augmentation du pourcentage de gras abdominal. L'augmentation de la teneur en lysine s'est traduite par une augmentation de la consommation alimentaire pour la période de démarrage, du gain de poids et de l'indice de consommation pour la période de croissance et l'ensemble de la période d'élevage et du rendement en filet à l'abattage. Les différents régimes alimentaires n'ont pas affecté la mortalité.

Introduction

Lysine (Lys) is accepted as the second limiting amino acid based on corn and soybean meal, therefore it will be possible to supplement poultry diet with crystalline Lys to reduce CP content of the diet. The NRC (1994) recommended that broilers receive 11.0, 10.0, and 8.5 g Lys/kg of diet at 0 to 3, 3 to 6, and 6 to 8 weeks of age respectively. Lys needed for optimizing breast meat yield may be higher than the amount needed for optimal BWG and feed efficiency (Jackson et al. 1989; Hickling et al., 1990; Moran et al., 1990; Acar et al., 1991; Gorman et al., 1995). Holshmier et al.(1993) showed that breast meat yield was increased in male broilers fed diet containing increasing Lys from 1-14 days of age, however the performance of broilers was not affected by dietary Lys from 15 to 49 days of age. It is well known that protein and Lys and its interaction is considered as an important factor which affects performance and carcass quality of growing chicks, and so, dietary requirement of protein is actually a requirement for the Lys contained in the protein. Therefore the objective of this study was to evaluate the Lys and protein levels effects on performance and carcass characteristics with recommended level protein and another diet lower in protein than the recommended level, without changing the Methionine+Cystine (Met+Cys), and Threonine requirements recommended by NRC (1994).

Materials and methods

An experiment with Ross 308 male broilers was conducted from 1 to 6 weeks of age. At day 1, 240 male chicks were placed in 24 floor pens (10 chicks per pen, and 0.1m^2 floor space/chicks). Feed and water were provided ad-libitum. Treatment diets consisted of

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three levels of L- Lys.HCl in starter and grower period (0.0, 1.5, and 3.0 g/kg diet), and two levels of protein (208.4, 178.4 in starter and 181.2, 161.2 in grower period) with 4 replicates/treatment, and 2900 kcal AME/kg diet. Treatments were derived by adding L-Lys.HCl (780 g L-Lys/kg) in basal diet. Prior to the experiment samples of ingredients (corn, soybean meal) were analyzed to determine protein and amino acid contents. Crude protein was calculated by Kjeldhalj method. Amino acid concentration was determined following acid hydrolysis, Met and Cys following performic acid oxidation using a high performance cation exchange column (AOAC, 1980).

Weight gain, feed consumption and feed efficiency (adjusted for mortality) were measured on a pen basis from 1 to 42 day period weekly. Mortality was recorded throughout the experiment. At 42 days of age 4 birds in each treatment (Close to pen means BW with no visible abnormalities) were killed by cervical dislocation for carcass characteristics. Prior to killing the birds, feed and water were withdrawn for 10 and 4 hr, respectively. For nitrogen excretion a balance trial was done. At 7 days of age a total of 74 male broiler chickens with similar pen weight distributed among 24 wire battery cages (3 in each pen), and fed the experimental diets for 10 days. Feed consumption and feces excretion were recorded from 18-21 days of age. Excreta were collected on polyethylene sheets placed under wire battery floor for 3 days. Samples were collected and then stored at -3° C until analyzing for nitrogen content. The factorial arrangement of 6 treatments consisting of two levels of CP and three levels of Lys with CRD design were analyzed using the General Linear Models procedure of SAS (SAS Institute, 1999). When differences among means were found, means were separated using Duncan's new multiple range test (Steel and Torrie, 1980).

Results and discussion

The results of broiler performance and carcass characteristics showed that reducing dietary protein decreased weight gain in starter, grower, and total period of the experiment up to 6.0, 4.6, and 4.6 % respectively ($P<0.05$). Lowering dietary protein also decreased feed consumption in starter period ($P<0.05$), but it had no significant effect on gain to feed ratio in all phases of the experiment. Decreasing dietary protein increased abdominal fat significantly ($P<0.05$). Increasing Lys level in the diet increased weight gain and gain to feed ratio in grower, and total period of the experiment ($P<0.05$). It also increased feed consumption in starter period of the experiment. A positive response in breast meat yield was achieved by increasing Lys level in the diet ($P<0.05$). The values of dietary Lys (11.2, and 12.4 in starter and 9.4, 10.6 g/kg in grower period) are higher than those of cited in the NRC (1994) based on 2900kcal AME/kg diet. The results confirmed the previous studies which demonstrated that Lys requirement for growing chicks is higher than that of which NRC (1994)

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recommendation which is supplemented on the diet for maximal growth. It is also confirmed that increasing dietary Lys level increases breast meat yield.

It is concluded that reducing dietary protein with adding 1.5 g L-Lys.HCl/kg diet in starter and grower period (based on 2900 kcal AME/kg diet) could be suitable for improving body weight gain, feed efficiency, and breast meat yield for Ross broiler chickens under the conditions of this study. It is apparent from this data that protein and Lys levels and their interactions had no significant effect on feed consumption in grower and total period of the experiment, showing that dietary treatment had not adverse effect on this trait. It was shown that feed consumption tended to increase slightly, as the protein and Lys level increased. It was clear from the data that birds utilized feed more efficiently in grower period. Dietary protein did not significantly affect the yield of breast meat and thigh meat ($P>0.05$).

There was a significant reduction in abdominal fat percentage for the higher protein diet (control). It might be a close relationship between fat percentage and the energy protein ratio. The smaller the ratio, the less fat will be deposited. Decreasing dietary protein increased the caloric protein ratio, resulting in excessive energy intake as relative to protein intake, thus resulted in higher carcass fat content, Although there was a trend for a reduction in fat pad percentage due to increase Lys level, the effect was not significant. Increasing Lys level in diet increased breast meat percentage significantly, as shown in other researches (Gorman et al, 1995; Han and Baker, 1994). The concentration of dietary Lys can significantly influence breast meat yield for several reasons: it contains a high concentration of Lys. Breast meat represents a large portion of carcass meat. Breast muscle development is also affected by sex, age, breed and genetics (Moran et al, 1990; Acar et al, 1991; Gorman et al, 1995). Their studies have also shown that an additional Lys increase breast meat accretion. Dietary Lys had not significant effect on mortality. nitrogen excretion was reduced significantly by decreasing dietary protein level (0.868 versus 0.758 g/bird/day). An attempt to reduce the protein level of the diet would help to decrease diet cost (depend on grain and oilseed meal cost). The results of the present study showed that protein level could be reduced approximately 30 in starter and 20 g/kg diet in grower period based on 2900 kcal AME/kg diet without adverse effect on gain to feed ratio. In this case additional amino acids would be added to the diet. By decreasing protein level in broiler diet nitrogen excretion will be reduced significantly ($P<0.05$).

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Table. Effects of dietary CP and Lys levels on broiler performance, carcass characterstics, and nitrogen excretion

Diet/traits	Weight gain (0-6 weeks)	Feed consumption (0-6 weeks)	Gain : feed (0-6 weeks)	Dressing percentage %	Breat meat yield (g)	Abdominal fat (%)	Nitrogen excretion (g/day/bird)
CP level							
Control	1755[a]	3522	0.498	64.23[a]	382.92	1.64[a]	0.868[a]
Low	1657[b]	3421	0.484	62.66[b]	375.08	2.22[b]	0.758[b]
Lys level							
0.00	1631[b]	3449	0.473[b]	64.11	320.63[c]	2.20	0.861
0.15	1735[a]	3455	0.502[ab]	63.68	410.00[a]	2.03	0.768
0.30	1757[a]	3511	0.499[a]	62.56	379.83[a]	1.56	0.808

Means in columns with different superscripts are significantly different (P<0.05).

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Session 6
Influence of production factors from farm to slaughter on meat and carcass quality

GENETIC VARIABILITY OF MEAT QUALITY

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Keywords : lines, genetic parameters, muscle fibre, pH fall, processing quality

Abstract

Results in chickens and turkeys on the within-and between lines genetic variability of technological quality of meat in relation with muscle fibre characteristics and post-mortem metabolism are presented. Even though no abnormal pH fall was observed, breast meat of birds selected for growth and breast yield was found paler, which may be undesired by the consumers. According to genetic parameters estimates, selection for meat quality is feasible, very significant heritabilities of meat traits being observed under highly controlled experimental conditions. However, large variations of the heritability estimates were reported, lower values being obtained for the thigh than for the breast characteristics or for meat traits measured under commercial than under experimental conditions. The latter point highlights the importance of limiting pre-slaughter stress by a good management of the birds, in order to optimize the response of selection for improved meat quality.

Résumé

Les résultats d'études de la variabilité entre souches et intra souche de la qualité technologique de la viande de poulet et de dinde en relation avec les caractéristiques des fibres et le métabolisme post-mortem des muscles sont présentés dans cet article. Même si aucune anomalie importante de la chute du pH dans le muscle après la mort n'est observée, les poulets sélectionnés pour la croissance et le développement musculaire présentent une viande plus pâle, tendance pouvant à terme déplaire aux consommateurs. Selon les premières estimations des paramètres génétiques, sélectionner sur la qualité technologique de la viande est possible, des héritabilités très significatives étant obtenues en conditions contrôlées. Cependant, le progrès génétique espéré peut être modulé par différents facteurs, tels que le type de muscle avec des h^2 plus faibles pour la cuisse que pour le filet, ou les conditions de pré-abattage avec des

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h^2 plus fortes en conditions expérimentales que commerciales, montrant l'importance d'une bonne maîtrise des conditions de pré-abattage limitant le stress.

Introduction

Poultry meat production has been very dynamic over the last decade and now occupies the second place in the world just after pork (Mandava and Hoogenkamp, 1999). At the same time, the marketing of poultry has been greatly diversified. For example in France, nearly 50% of the chickens are sold as parts or further processed products. This proportion should increase by 10% in the next five years (Magdelaine and Philippot, 2000). As a consequence, selection for edible meat yield, mainly breast yield, has intensified (Pollock, 1997) at least for standard production. If selection for growth and muscle development has been beneficial to production costs, its impact on meat quality is not well known. However, the continuing shift in the market from whole birds to further processed products has highlighted an increase in meat quality problems such as poor cohesiveness or water holding properties reported in turkeys (Sosnicki and Wilson, 1991) but also in chickens. By comparison to pigs, research on the relationships between muscle properties and meat quality has been limited in poultry. On the other hand, if the genetics of meat quality has been widely used in pig selection, including meat characteristics in poultry breeding is a much more recent focus. Studies about the genetic variability of muscle fibre characteristics and post-mortem muscle metabolism in relation with technological quality of chicken and turkey meat will be presented in the present paper. According to the state of the knowledge, results will concern between line or within line genetic variability of meat characteristics.

Selection for growth and muscle development: impact on muscle fibre characteristics

Experimental lines of chickens divergently selected for growth or muscle development are useful models to define better the effect of such selection on muscle fibre characteristics. Quantitative and qualitative properties of two metabolic types of muscles: the *Pectoralis major*, a pure fast twitch muscle, and the *anterior Latissimus dorsi* (ALD), a completely slow tonic muscle, were examined in an extreme model of chicken lines divergently selected for high or low body weight (1882 g *vs* 675 g at 77 days) (Rémignon *et al.*, 1995). Differences in muscle mass obtained by this selection appeared to be due to changes in both the number (with an estimated increase of 20%

in ALD) and muscle fibre diameter, which was twice as large in the fast growing birds than in the slow growing birds. As total number of fibres remained unchanged after hatching, this suggested that selection had influenced the *in ovo* development by increasing the fibre number in the muscles of the fast-growing birds. Post-hatch development was also modified, with a greater hypertrophy of muscle fibres from the latter birds. By contrast, whatever the muscle, selection for growth did not modify the myosin isoform profiles nor the activities of enzymes involved in the glycolytic (i.e. lactate dehydrogenase and citrate synthase) or the oxidative (i.e. β-hydroxyacyl coenzyme A dehydrogenase) metabolic pathway.

More recently, complementary studies were conducted to define the variations of muscle fibre characteristics between two chicken lines significantly differing for breast weight (205.0 g vs 150.4 g at day 42) but close for body weight (1484 g vs 1419 g) (Guernec *et al.*, 2003). The study of this model was aimed at estimating the specific effect on muscle fibres of the selection for higher breast yield, a major criterium of selection of the meat type strains. Chickens selected for high breast yield showed larger fibres than their controls in breast as well as in Sartorius muscle, a mixed oxidative and glycolytic leg muscle. The amplitude of this difference increased with age, fibres of the selected birds being 24% larger at 6 wks of age. In contrast, estimation of fibre number index in breast did not differ between both genotypes. Moreover, the activity of the enzymes used as markers of glycolytic and oxidative pathways was not significantly modified (Berri *et al.*, 2001).

Results obtained up to now in chickens lead to the consensual conclusion that the increased breast muscle mass obtained by selection has mainly been achieved by a muscle fibre hypertrophy. In contrast, the metabolic pattern of breast muscle seems rather insensitive to such selection. This is probably related to the fact that this muscle is composed only of white glycolytic fibres (Rémignon et al., 1996), which does not allow a great plasticity of the metabolic pattern. Further investigations are now needed to detail to what extent the changes in fibre size brought by selection have modified the quality of the meat.

Genetic variability of post-mortem muscle metabolism in relation with processing quality of meat.

In poultry as in pigs, variations in the onset of *rigor mortis* significantly affect the storage and processing quality of meat. As reported by Offer (1991), a rapid post-mortem pH decline may affect the color, texture and water-holding capacity of meat as largely described for the PSE syndrome in pigs. On the other hand, a low final pH leads to a pale aspect and a low water-holding capacity of chicken (Barbut, 1997) and turkey meat (Barbut, 1996). Whereas, meat with a high final pH is known as dry, firm

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and dark (DFD) and presents a poor storage quality due to a faster rate of off-odor production and accelerated microbiological growth (Allen *et al.*, 1997). Genetic variability of the onset of the *rigor mortis* was suggested by studies comparing various chicken lines. Differences in the rate of pH fall between five commercial chicken lines were reported (Gardzielewska *et al*., 1995), but no attempt was made to relate these observations to variations in growth performances or muscle metabolism. Results of some recent studies aimed at comparing post-mortem muscle metabolism and meat quality of various chicken or turkey lines differing for growth and muscle development will be presented. First estimates of the genetic parameters of meat quality traits in both species will also be detailed.

Between line variability

In a recent study, Berri *et al.* (2001) compared breast meat metabolism and meat quality of four chicken lines: an experimental and a commercial line selected for increased body weight and breast yield, and their respective unselected control lines. Birds from the experimental selected (ES) line exhibited similar body weight but higher breast yield (+21%) and lower abdominal fat percentage (-25%) than their controls (EC). Breast and fat yields of the birds from the commercial selected (CS) line were higher (+61% and +18%, respectively) than those of the commercial control (CC) line. Very similar trends were observed in both the experimental and commercial models. A significant effect of the line on the initial rate of pH fall was observed, pH drop being delayed in breast of the selected birds by comparison to their controls. At the same time, the extent of the pH fall was less pronounced in the selected birds which presented a higher ultimate pH than their controls. This could be due to the lower glycolytic potential of the selected birds, as final pH is known to be mostly dependent on the initial glycogen reserves of muscle at the time of slaughter (Bendall, 1973). Despite a lower initial pH decline and a higher final pH, breast meat of the selected birds was lighter and less red, probably because of a lower heme pigment content. On the other hand, the color modification induced by selection was not associated with an excessive drip loss of breast meat of the selected birds (no more than 1.75% after 5 days of storage).

A similar approach was conducted by Fernandez *et al.* (2001) in turkeys, by comparing two genetic types differing for growth, *i.e.* a standard fast-growing (FG) line and a "label" slow-growing (SG) line, and the product of their crossbred. Post-mortem metabolism and meat quality of breast (*Pectoralis major*) and thigh (*Iliotibialis*) muscles were measured. In accordance with previous works in the chicken, no differences in the muscle enzyme activities were observed in relation with the genetic background of the animals (Rémignon *et al.*, 2000). However, FG birds exhibited a lower glycogen content in the *Pectoralis major* muscle than SG birds, the crossbred being intermediate, while the reverse situation was found in the *Iliotibialis* muscle. Despite these differences in glycogen content at slaughter, there was no difference in the ultimate pH of the *Pectoralis major* and *Iliotibialis* muscles between

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the three genetic types. On average, the initial rate of pH fall in the *Pectoralis major* muscle was similar in the three genotypes, but the frequency of birds with a low pH at 20 min post-mortem was greater in the slow-growing line. This suggested a lower homogeneity of breast meat quality of the SG birds. The latter also exhibited a faster rate of pH fall in the *Iliotibialis* muscle by comparison to FG birds. This effect was however of low magnitude as pH values at 20 min post-mortem remained high. Some differences in meat quality indicators were observed between the three genotypes. By comparison to FG birds, SG birds exhibited higher drip loss but lower curing-cooking of breast meat.

No evidence of PSE defect was observed in these two studies, even for the most intensively selected birds. This could partly be related to the highly controlled slaughter conditions used in these experiments, that were rather far from commercial more stressful conditions. As reported in the review of Sosnicki et al. (1998), even if the ultimate cause of the PSE phenomenon is not known, it appears to be as in pigs, the result of the combination of stress sensitivity and of the predominant glycolytic metabolism of breast muscle. On the other hand, even though no abnormal pH fall was observed, breast meat of the selected birds was found paler, which may be undesired by the consumers. Impact of the larger fibre size (increasing color reflectance) and of the lower pigment content (decreasing redness) of their muscles was suspected. Moreover, a depletion of glycogen content at slaughter in breast of the selected birds was observed. As discussed by Fernandez et al. (2001), the question remains as to whether this could be due to differences in the muscle glycogen reserves or to stress-induced glycogen depletion before slaughter in the selected birds, which are generally assumed to be more stress sensitive.

Genetic parameters estimates

A genetic study of several indicators of breast meat quality was conducted by Le Bihan-Duval *et al*. (2001)in an experimental broiler line. Birds, slaughtered in an experimental processing plant, did not undergo transport before killing. The measurement of the ultimate pH and the color variables (L*: lightness ; a*: redness ; b*: yellowness) were recorded on a total of 1076 birds. The pH at 15 min post-mortem and the drip loss of the meat were also recorded for some of the animals (about 600). REML estimates indicated that meat traits had very significant heritabilities, the highest values being obtained for the color parameters (Table 1).

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Table 1. Estimates of genetic parameters[A] for breast meat quality and growth performances[B] in a broiler chicken line (Le Bihan-Duval *et al.*, 2001).

	pH_{15}	pHu	L*	a*	b*	DL	BW	BRY	AFP
pH_{15}	**0.49**	0.02	0.13	-0.23	0.05	-0.29	-0.06	0.12	-0.04
pHu		**0.35**	-0.91	0.14	-0.43	-0.83	0.07	0.13	-0.54
*L**			**0.50**	-0.48	0.20	0.80	0.16	-0.07	0.50
a*				**0.57**	0.54	-0.25	-0.30	-0.29	-0.24
b*					**0.55**	0.16	-0.13	-0.39	-0.02
DL						**0.39**	-0.04	-0.16	0.29
BW							**0.35**	0.17	0.19
BRY								**0.55**	-0.17
AFP									**0.62**

[A] Heritabilities on the diagonal, genetic correlations above the diagonal.

[B] pH_{15} = pH 15 at min post-mortem; pHu = ultimate pH; L* = lightness; a* = redness; b* = yellowness; DL = drip loss; BW = body weight; BRY = breast yield; AFP = abdominal fat percentage.

The estimated genetic correlation between pH at 15 min post-mortem and ultimate pH was found to be equal to zero, showing that the rate and the extent of the post-mortem pH fall were under different physiological controls. This result agreed with the fact that the ultimate pH of the meat is supposed to be mostly dependent on the initial glycogen reserves of the muscle at the time of slaughter, whereas the rate of the pH fall is related to the glycolytic enzyme activities just after death. The ultimate pH appeared as a determining factor of meat quality, as significant negative correlations were found between this trait and the lightness and drip loss of the meat. By contrast, pH at 15 min post-mortem was not genetically correlated with the other quality indicators. Body weight and breast yield were poorly genetically related with the degree of pH fall, while moderate negative genetic correlations were observed with the redness and yellowness of the meat. This suggested that selection for growth and muscle development would not alter the meat pH, but could slowly modify its color by decreasing the redness and yellowness indicators. The experimental conditions used for this study ensured a very accurate control of the meat traits measurements, that may have contributed to the high level of heritability estimates. However, they did not allow real defects of meat quality (especially PSE meat) to be observed, as would be expected under industrial more stressful conditions.

A second study, carried out in turkeys, was conducted under industrial conditions. This study, conducted in a grand-parental female turkey line (BUT Ltd), was aimed at (1) detailing the effect of post-mortem pH decline rate on the processing ability of meat and (2) estimating the genetic parameters of pH at 20 min post-mortem, ultimate pH and meat color under industrial conditions. On the day of slaughter, the distributions of the pH at 20 min post-mortem (pH_{20}) were established for both the *Pectoralis*

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superficialis (PS) muscle (breast muscle) and *Iliotibialis* (IL) muscle (thigh muscle). This allowed the meat to be separated in three or two groups for the breast and thigh muscles, respectively, according to the pH_{20} level. The PS and IT muscles were further processed in LDC commercial plant (Sablé – France) into white cured-cooked meat and turkey ham, respectively. The meat was processed according to "high-quality" French standards (without use of polyphosphates) in order not to hide the effect of the quality of the raw meat. As reported by Fernandez *et al.* (2002), the technological yield was significantly lower in the groups showing the lowest pH_{20} for both the white meat and the ham. Lower pH_{20} values were also associated with increased drip loss and a paler aspect (higher lightness) of the meat. REML estimates of the genetic parameters for pH_{20}, ultimate pH and color indicators (L*; a*; b*) were calculated, on all the available 420 pedigree birds. These birds were the progeny of 30 sires and 118 dams. Moderate levels of heritability were obtained for pH_{20} measured in the breast (h^2= 0.21) (Table 2) and in the thigh muscle (h^2 = 0.20).

Table 2. Estimates of genetic parameters[A] for breast meat quality and growth performances[B] in a commercial turkey line (Le Bihan-Duval *et al.*, 2003).

	BW	BRY	PH_{20}	pHu	L*	a*	b*
BW	**0.35**	0.11	0.55	0.55	-0.41	0.13	-0.49
BRY		**0.32**	0.62	0.23	-0.24	-0.18	-0.31
PH_{20}			**0.21**	0.59	-0.80	-0.25	-0.35
pHu				**0.16**	-0.53	0.08	-0.15
L*					**0.12**	0.22	0.54
a*						**0.21**	-0.09
b*							**0.14**

[A] Heritabilities on the diagonal, genetic correlations above the diagonal.
[B]BW = body weight; BRY = breast yield; pH_{20} = pH at 20 min post-mortem; pHu = ultimate pH; L* = lightness; a* = redness; b* = yellowness.

Other meat characteristics exhibited low to moderate heritabilities, ranging from 0.12 to 0.21, when measured in breast muscle. By contrast, the impact of genetics appeared extremely low for the same traits measured in thigh muscle, with heritabilities close to zero. To our knowledge, no other genetic parameters of thigh meat characteristics have been published in poultry. However, in the study of Larzul (1997) in pigs very low heritability of L* and a* values were also obtained in one red muscle, by contrast with the intermediate values found in some mixed and white muscles. Our results indicated a strong negative genetic correlation between pH_{20} and the lightness of breast meat. This could be explained by the fact that both traits are characterizing the PSE syndrome, abnormally low pH at an early post-mortem time, when carcass temperature is still high, leading to protein denaturation responsible for color (and water-binding capacity) alterations. Surprisingly, a significant positive genetic correlation was

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estimated between the rate and the extent of the pH fall in breast muscle. No explanation can be given at this time, as this was not consistent with our first results in chickens nor with the genetic results obtained in pigs. According to our results, the lowest pH values, either at 20 min and 24 h post-mortem, were associated with the lowest growth and muscle development performances, with very significant positive genetic correlations. These results did not confirm the hypothesis that birds intensively selected for growth and muscle development would be more susceptible to meat defects. On the other hand, this contrasted with the results obtained in pigs, for which a genetic antagonism between technological quality and growth or body composition traits has often been reported (Sellier and Monin, 1994). However, we must highlight the fact that HAL and RN major genes in segregation in the pig populations are strongly responsible for this antagonism, while their impact in poultry lines has not been proved yet.

Conclusion

The genetic results obtained up to now indicate that selection for meat quality is feasible, very significant heritabilities of breast meat traits being observed under highly controlled experimental conditions. However, as meat quality is a complex trait, efficiency of selection could be modulated by several factors, intrinsic (such as the type of muscle) or extrinsic (such as pre-slaughter conditions) to the animal. The few available results in the turkey indicate small heritabilities of thigh meat characteristics, showing that selection for thigh meat quality would be poorly efficient. In addition, important interaction between genotype and pre-slaughter condition was suspected, excessive rate of pH fall being observed only under industrial more stressful conditions. This highlighted the importance of limiting pre-slaughter stress by a good management of the birds, in order to optimize the response of selection for improved meat quality. Moreover, as measuring meat quality remains laborious and often requires the birds to be killed, finding genetic markers could enable selection methods to be improved.

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CONTENTS

PRE-SLAUGHTER ALTERATIONS IN BLOOD CHEMISTRY OF BROILER CHICKENS

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Keywords : Pre-slaughter, broiler chickens, blood chemistry

Abstract

A close coordination is essential between the live production and processing phases within an integrated broiler production system to maintain a steady supply of live birds to the processing plant. In this study, blood chemistry of male and female broiler chickens from two diverse strain-crosses (fast-food and yield type) subjected to pre-slaughter transportation was assessed at 39 and 53d of age. At each age, feed was withdrawn 4 h prior to catching and cooping. One-half of the coops (by strain-cross, sex, and replicate pen) were either held stationary for 10h, or subjected to a 6h transportation followed by a 4h of stationary holding prior to slaughter. Blood chemistry profiles showed few differences due to age, strain-cross and sex. However, significant treatment effects were detected from transportation due to increased dehydration, hemoconcentration and protein catabolism.

Résumé

Dans un système intégré de production de poulets, une bonne coordination des phases de production et d'abattage est essentielle pour maintenir un apport constant d'animaux à l'abattoir. dans cette étude, la composition du sang était analysée à 39 et 53 jours d'âge chez des poulets mâles et femelles issus de deux croisements (fast-food et rendement) et soumis à un transport avant l'abattage. La nourriture était retirée 4 h avant le ramassage et les animaux transportés à l'abattoir. Une moitié des cages de transport stationnait à l'abattoir pendant 10 h, tandis que l'autre moitié était transportée pendant 6 h, puis stationnait 4 h avant l'abattage. (dispositif équilibré pour les croisement, sexe et case d'élevage). Les profils de composition du sang montraient peu de différence entre âges, sexes et croisements. Cependant le transport entaînait une hémoconcentration et augmentait le catabolisme protéique.

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Introduction

Market age broilers must be transported to the processing plant. To assure a steady and uninterrupted supply, a close coordination is essential between the production and plant personnel. In scheduling flocks for transportation, many factors must be taken into account, including feed withdrawal program, farm location (distance), catching time (day or night) and method (speed), weather conditions, plant holding capacity, and processing line-speed (birds per h). Minimizing stressors during this short pre-slaughter phase is essential to reduce the likelihood for mortality (DOA), weight loss (shrink) and carcass and meat quality problems (Freeman *et al.,* 1984 ; Kettlewell, 1989 ; Nico and Scott, 1990 ; Moran and Bilgili, 1995).
Freeman (1984) outlined several potential stressors during this period, including handling, inversion, crating, confinement, social disruption, changes in micro and macro climate, fasting, air movement, vibration, and noise. The purpose of this study was to compare the changes in blood profile of broilers either held stationary or subjected to active transportation, followed by a period of rest, prior to processing.

Material and Methods

Male and female broilers from two diverse strain-crosses (SC ; fast-food and yield type) were reared to 53 d of age (four replicate pens per sex and SC ; 16 pens total) on common feeding and management conditions. Both at 39 and 53 d of age, feed was removed for 4 h (with water available) prior to catching. Seven birds were placed per coop (four marked coops per pen) for transport to the Pilot Processing Plant. One-half of the coops from each pen were placed on an open-bed trailer for transporartion (TR), whereas the other half were held stationary (SH) at the plant for 10h. Transportation treatment was 6h in length, followed by a 4 h rest prior to processing. Coops from each pen was processed sequentially, with alternating TR and SH treatments. Birds were electically stunned in water-bath stunner, and manually cut for exsanguination. Blood (5 ml) was collected from each bird into marked individual plastic tubes (10 birds per treatment per pen). Blood was allowed to clot and serum was separated by centrifugation (Spano *et al.,* 1987). One ml of serum was pooled by treatment from each pen (32 pooled samples per age) and used for blood chemistry. Serum samples were then analyzed in duplicate by a Cobas Mira Chemistry Analyzer, using commercially available kits for glucose, total protein, uric acid, sodium, chloride, calcium, potassium, phosphorus, aspartate transferase (AST), creatine phosphokinase (CPK), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), and serum alkaline phosphatase (SAP). Data were analyzed, by age, as a 2x2x2 factorial

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arrangement of SC, sex, and transportation treatments by the General Linear Models Procedure of SAS (1988).

Results and Discussion

Select serum chemistry values of broiler chickens are presented by SC, sex, transportation treatments, and age in Tables 1-3. It is difficult to obtain baseline clinical chemistry values for broiler chickens, as these values have been shown to vary extensively with genetics (i.e., layer *vs.* broiler lines), feeding/fasting status, micro/macro climate, age, sex, physiological state, health status, as well as methods used in blood sampling and analysis (Bowes *et al.,* 1989). Tables 1-3 summarize the blood chemistry of broilers by main effects of SC, sex, and transportation treatment at both market ages. There were no significant two- or three-way interactions in this study ($P>0.05$). Few differences were observed in blood chemistry values due to SC (GGT at 6 wk and SAP at 8 wk of age), and sex (sodium and potassium at 6 wk and SAP at 6 and 8 wk of age). On the other hand, significant increases in serum uric acid, sodium, chloride, potassium, AST, CPK, and LDH , and decreases in serum phosphorus, GGT and SAP were detected for the TR treatment at both ages. Serum AST, CPK, and LDH levels appeared to increase with age, whereas the glucose and SAP levels decreased from 39 to 53 d of age. Age related drop in serum glucose values in this sttudy was unexpected and unexplanable. However, values this low have been reported in birds with spiking mortality syndrome (Davis and Vasilatos-Younken, 1995) and with mycotoxicosis (Espada *et al.,* 1994).

Blood chemistry profiles obtained indicates that long transportation period (6 h) may be a stress factor in itself in pre-slaughter broilers. Subsequent rest period of 4h did not stabilize the effects of transportation in this study. Increases in serum enzyme levels, particularly CPK and LDH, are attributed to tissue damage ; changes in uric acid, GGT and AST to alterations in energy metabolism (lipolysis and gluconeogenesis); and increases in serum electrolytes to hemoconcentration (dehydration). These alterations in blood chemsitry are consistent with those reported in the literature for transported broilers (Halliday *et al.,* 1977 ; Freeman *et al.,* 1984 ; Cashman *et al.,* 1989 ; Duncan, 1989 ; Mitchell *et al.,* 1992 ; Yalcin *et al.,* 2001).

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Table 1. Select serum chemistry values in transported broiler chickens

	Glucose		Total Protein		Uric Acid	
	39d	53d	39d	53d	39d	53d
	----(mg%)----		----(g%)----		-----(mg%)-----	
Strain Cross[1]	NS	NS	NS	NS	NS	NS
FF	212	108	3.53	3.70	6.36	7.42
YT	206	115	3.48	3.66	6.38	6.42
Sex	NS	NS	NS	NS	NS	NS
Female	209	118	3.48	3.70	6.30	6.77
Male	208	106	3.53	3.66	6.44	7.07
Transportation[2]	NS	NS	NS	NS	*	***
SH	206	120	3.49	3.73	5.11	5.11
TR	212	104	3.51	3.63	7.63	8.73
SEM[3]	3.2	6.9	0.04	0.04	0.68	0.58

[1]FF = fast-food; YT = yield type
[2]SH = Stationary holding; TR = Transported
[3]Pooled standard error of the mean.
NS = Not significant (P> 0.05); *P < 0.05; *** P < 0.001

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Table 2. Select serum chemistry values in transported broiler chickens.

	Sodium		Potassium		Chloride		Calcium		Phosphorus	
Factor	39d	53d	39d	53d	39d	53d	39d	53d	39d	53d
	----------(mmol/L)----------						----------(mg%)---------			
Strain Cross[1]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
FF	153	160	8.6	7.9	117	117	8.4	9.0	7.4	8.3
YT	152	160	8.6	8.0	116	117	8.2	8.5	7.4	7.8
Sex	*	NS	**	NS	NS	NS	NS	NS	NS	NS
Female	152	160	8.8	8.0	117	117	8.2	8.6	7.4	7.8
Male	153	160	8.3	7.9	116	116	8.4	8.8	7.4	8.3
Transport[2]	***	***	NS	***	NS	***	NS	NS	***	*
SH	151	157	8.5	7.4	116	115	8.1	8.9	7.6	8.4
TR	154	163	8.6	8.5	117	118	8.4	8.5	7.1	7.7
SEM[3]	0.5	0.7	0.1	0.2	0.3	0.5	0.2	0.2	0.1	0.1

[1]FF = fast-food; YT = yield type
[2]SH = Stationary holding; TR = Transported
[3]Pooled standard error of the mean.
NS = Not significant (P> 0.05); *P < 0.05;**P < 0.01; *** P < 0.001

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Table 3. Select serum chemistry values in transported broiler chickens (IU/L).

	AST[4]		CPK[5]		GGT[6]		LDH[7]		SAP[8]	
Factor	39d	53d	39d	53d	39d	53d	39d	53d	39d	53d
Strain Cross[1]	NS	NS	NS	NS	*	NS	NS	NS	NS	**
FF	324	412	10647	18727	19	21	3667	4643	4356	1475
YT	323	417	10033	19748	21	21	3694	4760	4460	1153
Sex	NS	NS	NS	NS	NS	NS	NS	NS	***	NS
Female	325	423	10486	19928	20	22	3592	4655	3489	1220
Male	322	406	10195	18547	20	20	3768	4748	5327	1408
Transport[2]	**	***	**	***	*	***	***	***	*	**
SH	309	362	8918	12316	21	24	3418	4261	4707	1454
TR	338	467	11762	26159	19	18	3943	5141	4109	1174
SEM[3]	6.0	13	575	1912	0.5	0.9	97	128	185	66

[1]FF = fast-food; YT = yield type
[2]SH = Stationary holding; TR = Transported
[3]Pooled standard error of the mean.
[4]Aspartate amino transferase
[5]Creatine phosphokinase
[6]Gamma glutamyl transferase
[7]Lactate dehydrogenase
[8]Serum alkaline phosphatase.
NS = Not significant (P> 0.05); *P < 0.05; **P < 0.01; *** P < 0.001

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BETWEEN LINE VARIABILITY OF CHICKEN TECHNOLOGICAL MEAT QUALITY UNDER DIFFERENT PRE-SLAUGHTER CONDITIONS.

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Keywords : Chicken, Genotype, Meat Quality, Pre-slaughter Stress, Behaviour

Abstract

This study aimed at defining the variability of chicken technological meat quality among several genotypes, in relation with muscle characteristics and stress reactivity measured under different pre-slaughter conditions. A slow-growing label grand-parental line, a fast-growing standard grand parental line and a male line highly selected for breast yield were compared after these birds were subjected either to a hanging stress, to a heat + hanging stress or killed under control conditions limiting stress. Irrespectively of the line, hanging stress led to a faster rate of pH drop, while heat stress affected ultimate pH only. Behaviour on shackle line largely varied among genotypes, slow-growing birds appearing as the most active birds. This could at least partly explain the observed between-line variability of some meat quality indicators and the interaction with pre-slaughter condition, the most active birds being more penalized by hanging condition. On the other hand, implication of structural characteristics of the breast muscle of the high breast yield birds on the pale aspect and low water-holding capacity of their meat was suspected.

Résumé

Cette étude visait à estimer la variabilité de la qualité de la viande en relation avec les caractéristiques musculaires et la réactivité au stress entre poulets issus d'une souche grand-parentale label à croissance lente, standard à croissance rapide ou sélectionnée sur le développement musculaire placés face à différentes conditions de pré-abattage.

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Avant leur mort, les poulets étaient soumis soit à un stress d'accrochage, à un stress thermique puis d'accrochage, ou placés dans des conditions témoins minimisant le stress. Quelle que soit la lignée, le stress "accrochage" entraînait une augmentation de la vitesse de chute du pH, alors que la chaleur affectait le pH ultime. Cette étude révélait une forte variabilité de l'activité sur la chaîne d'abattage entre les génotypes; celle-ci pouvait en partie au moins expliquer la variabilité entre souches de certains indicateurs de qualité et les interactions avec les conditions de pré-abattage, les animaux les plus actifs étant pénalisés par le stress d'accrochage. Enfin un effet des caractéristiques structurales du filet des animaux à fort développement musculaire sur l'aspect pâle et le faible pouvoir de rétention en eau de leur viande était suspecté.

Introduction

In France, nearly 50% of the chickens were sold as joints or further processed products in 1998 (Magdelaine and Philippot, 2000). This shows the importance of controlling the technological meat quality for this industry. The genetic variability of poultry technological meat quality has already been investigated (Berri *et al*. (2001) and Le Bihan-Duval *et al.* (2001) for chickens, Le Bihan-Duval *et al.* (2003) for turkeys), however, the impact of pre-slaughter conditions on meat quality was not clearly defined in these studies. Therefore, the present study aimed at estimating the between-line variability for chicken breast meat quality measured under different experimental pre-slaughter conditions reproducing varying stresses likely to occur under commercial conditions. A slow-growing grand-parental line, a fast-growing grand-parental line and a male line highly selected for breast yield were compared after birds were subjected either to a hanging stress or to a heat + hanging stress or killed under control conditions, limiting pre-slaughter stress.

Material and Methods

Animals. Female chickens from three grand-parental lines corresponding to a slow-growing label line (SG), a fast-growing standard line (FG) and a male line highly selected for breast yield (BY) were used. A total of 90 birds per line were slaughtered at their respective market age of 6 weeks for FG and BY lines and 12 weeks for SG line.

Pre-slaughter conditions: Animals were subjected before slaughter either to a “hanging” stress during which animals could struggle freely during 2 min on a moving shackle line, or to a “heat plus hanging” stress during which animals were first placed

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in a room at 35°C during 3 h 30 min and then hung on a moving line during 2 min. A control group was also constituted by hanging birds just before electrical stunning to minimize struggle on the shackle line. These two stressing pre-slaughter conditions were chosen because a negative impact of free struggle and heat stress on meat quality had previously been suggested by Debut *et al.* (2003).

Behavioural measurements. Stress reactivity was estimated by the level of activity on the shackle line measured by several criteria: presence or absence of straightening up (SU) of the birds (head over legs) recorded from hanging to stunning, vocalisations (VOCAL) classified into 4 categories from 0 when the bird did not shout to 3 when the bird screamed strongly during a long time, initial duration of wing flapping (IDWF) and total duration of wing flapping (TDWF) recorded in seconds respectively at the time of hanging or from hanging to stunning of the birds and then categorized into 3 classes of equal size. Tonic immobility (TI) tests were carried out one week before slaughter to estimate the basal level of emotivity of the birds.

Muscle characteristics and meat quality measurements. The pH at 15 min (pH15min), the ultimate pH (pHu) at 24h *post-mortem*, the colour at 24h *post-mortem* (lightness (L*), redness (a*) and yellowness (b*)) and drip loss (DL) at 72h *post mortem* were measured on right breast muscle (*Pectoralis major*) as described in Le Bihan-Duval *et al.* (1999). The curing-cooking yield (CCY) were measured as described in Naveau (1985) on the left breast taken at 24h *post-mortem.*

Statistical analyses. Relationships between muscle characteristics and meat quality traits were analysed within each line by a Principal Component Analysis (PCA) using the SPAD 4.0 software (Cisia, Montreuil, France). Genotype and pre-slaughter condition effects and their possible interaction on muscle characteristics and meat quality indicators were tested by a two-way ANOVA using the GLM procedure of SAS (SAS Institute, 1989). Pairewise comparison of means for each significant effect of the ANOVA were performed by Scheffe test using LSMeans statement of GLM procedure. Effect of the genotype on categorical behavioural variables were tested using Chi-square test of the FREQ procedure of SAS.

Results and discussion

The first PCA axis was mainly explained by the variation in the ultimate pH which was highly correlated with lightness, curing-cooking yield and drip loss. Indeed, correlations of -0.71, -0.67 and -0.51 were found between pHu and L* in SG, FG and BY lines, respectively. Significant negative correlations were also observed between pHu and DL in breast with r values of -0.58, -0.53 and -0.38 in SG, FG and BY lines, respectively. The intensity of the relationship between pHu and CCY varied according to genotype, the correlation being stronger (+0.71) for SG birds than for the two other

genotypes (+0.26 and +0.29 for FG and BY, respectively). The second PCA axis was mostly explained by the variations in pH15min as well as a* and b* values of breast muscle. The correlations between pH15min and a* were estimated at -0.32, -0.24 and -0.21 for the SG, FG and BY birds, respectively. Significant correlations of 0.21 between pH15min and b* and of -0.35 between pH15min and DL were obtained for SG birds only.

As reported in Table 1, a highly significant effect of genotype was observed for all behavioural measurements of activity on the shackle line while no effect was detected for tonic immobility test (data not shown). BY birds could be distinguished from both other genotypes by a much lower frequency of SU events and shorter IDWF and TDWF values. Distinction between FG and SG birds was mostly done on IDWF and TDWF values, which were longer for the first genotype while little difference was observed for SU frequency. Significant differences were also observed for vocalisations, but no clear tendency could be distinguished. In summary, on the basis of struggle activity on the shackle line, SG birds appeared as the most reactive birds, BY birds as the less reactive ones while FG birds could be considered as intermediate. This could be quantified among others by the IDWF values estimated at 6.4, 3.8 and 2.4 s for SG, FG and BY birds, respectively.

As shown in Table 2, *post-mortem* pH fall significantly varied among genotypes, SG birds exhibiting the lowest pH15min values and BY birds the highest. Similarly, significant differences were observed for ultimate pH, SG birds showing the lowest values and FG birds the highest. A significant interaction between line and pre-slaughter condition was revealed for L*. Breast colour significantly varied between genotypes, BY birds exhibiting the highest L* and the lowest a* values while SG birds were characterized by the highest a* and b* values. Pre-slaughter conditions significantly affected breast pH15min, pHu and a* values. By comparison to the control condition, pH15min was significantly decreased and a* increased by "hanging" stress while no specific effect of heat stress was observed. In contrast, pHu appeared to be mostly affected by heat stress, as only "heat + hanging" condition led to a significant decrease of this parameter in comparison with the control condition. Significant interactions between line and pre-slaughter condition were further observed for DL and CCY. While SG birds appeared to be affected by struggle on the shackle line, the other genotypes were less sensitive. Indeed, by comparison to the control condition, after hanging, DL increased (from 0.5%) and CCY decreased (from 2%) for SG birds while they were little modified for FG birds or even slightly improved for BY birds.

In conclusion, the present study revealed that behaviour on shackle line varied strongly across genotypes, SG birds appearing more active. The difference in pre-slaughter activity may at least partly explain the observed between-line differences in some meat quality indicators and line x pre-slaughter condition interactions, as the impact of hanging may be larger in more active birds. Structural differences in breast muscle of

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the BY birds may also explain the pale aspect and low water-holding capacity of their meat.

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Table 1. Repartition of the birds of each line (SG : slow-growing line, FG : fast-growing line BY : high breast yield line) into the classes of behavioural measurements and Chi-square probabilities.

	Straightening up		Vocalisations				Initial duration of wing flapping			Total duration of wing flapping		
	0	1	0	1	2	3	0	1	2	0	1	2
BY	72.73	27.27	26.32	16.84	44.21	12.63	58.59	26.26	15.15	41.41	36.36	22.22
FG	45.83	54.17	21.51	21.51	37.63	19.35	25.51	55.10	19.39	19.39	55.10	25.51
SG	49.49	50.51	35.87	16.30	11.90	35.87	46.46	5.05	48.48	30.30	13.13	56.57
Chi-square probability	0.0002		<.0001				<.0001			<.0001		

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Table 2. Means and standard deviations (in brackets) for muscle characteristics and meat quality traits of breast muscle for each line (SG : slow-growing line, FG : fast-growing line BY : high breast yield line) and probabilities of the ANOVA.

	Mean (SD)			Probabilities of the ANOVA			LSMeans[β]	
	SG (A)	FG (B)	BY (C)	Line effect	Pre-slaughter condition effect	Line* pre-slaughter condition interaction	Line	Pre-slaughter condition
pH at 15 min								
Control (1)	6.39 (0.15)	6.49 (0.11)	6.57 (0.12)				(A) 6.32 [c]	(1) 6.48 [a]
Hanging (2)	6.29 (0.19)	6.45 (0.10)	6.51 (0.11)	***	***	NS	(B) 6.45 [b]	(2) 6.42 [b]
Hanging + Heat (3)	6.29 (0.17)	6.43 (0.09)	6.50 (0.12)				(C) 6.53 [a]	(3) 6.40 [b]
Ultimate pH								
Control (1)	5.70 (0.10)	5.81 (0.11)	5.68 (0.13)				(A) 5.66 [c]	(1) 5.73 [a]
Hanging(2)	5.65 (0.12)	5.79 (0.11)	5.73 (0.11)	***	*	NS	(B)5.79 [a]	(2) 5.72 [ab]
Hanging + Heat(3)	5.62 (0.09)	5.76 (0.11)	5.68 (0.10)				(C) 5.70 [b]	(3) 5.69 [b]
Lightness (L*)								
Control (1)	50.5 (2.5)	51.6 (2.2)	54.6 (2.5)					
Hanging (2)	50.7 (2.8)	52.4 (2.4)	53.4 (2.9)	***	NS	*		
Hanging + Heat (3)	51.8 (3.4)	51.7 (2.5)	53.7 (2.3)					
Redness (a*)								
Control (1)	0.77 (0.67)	0.08 (0.68)	-0.59 (0.84)				(A) 0.96 [a]	(1) 0.09 [b]
Hanging (2)	1.34 (1.09)	0.24 (0.83)	-0.30 (0.68)	***	*	NS	(B) 0.20 [b]	(2) 0.43 [a]
Hanging + Heat (3)	0.78 (0.71)	0.29 (0.55)	-0.22 (1.0)				(C) –0.37 [c]	(3) 0.29 [ab]
Yellowness (b*)								
Control (1)	11.6 (1.5)	9.1 (1.2)	8.5 (1.6)				(A) 11.3 [a]	(1) 9.7
Hanging (2)	11.2 (1.9)	9.0 (1.3)	8.8 (1.2)	***	NS	NS	(B) 9.1 [b]	(2) 9.7
Hanging + Heat (3)	11.1 (1.4)	9.2 (1.4)	8.8 (1.6)				(C) 8.7 [b]	(3) 9.7
Drip loss (%)								
Control (1)	1.0 (0.4) [a 1]	1.4 (0.7) [ab 1]	2.0 (1.1) [b 1]					
Hanging (2)	1.5 (0.8) [a 1]	1.4 (0.8) [a 1]	1.5 (1.0) [a 1]	***	NS	**		
Hanging + Heat (3)	1.3 (0.6) [a 1]	1.2 (0.6) [a 1]	1.5 (0.7) [a 1]					
Curing cooking yield (%)								
Control (1)	84.0 (3.0) [a 1]	84.6 (2.8) [a 1]	82.1 (2.8) [a 1]					
Hanging (2)	82.0 (3.3) [a 1]	84.1 (2.2) [a 1]	82.9 (3.9) [a 1]	***	NS	**		
Hanging + Heat (3)	81.5 (2.9) [b 1]	85.1 (3.2) [a 1]	83.2 (3.0) [ab 1]					

[β] only given in absence of interaction between line and pre-slaughter condition. LSMeans with different letters within a column differ significantly ($P<0.05$).
*** $P<10^{-3}$; ** $P<10^{-2}$; * $P<5\times10^{-2}$; NS : non significant at 5%.

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RESPONSE OF BREAST MEAT TO PRESLAUGHTER BROILER HANDLING AND DELAYED CARCASS DEBONING

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Keywords : breast meat yield, carcass processing, myopathy, preslaughter stress

Abstract

Broilers received either "normal" preslaughter stress or avoided carrying-transport. Resultant carcasses were slush-ice chilled either 6 or 30 hours then breast meat removed. Similar yields of carcass and abdominal fat occurred between preslaughter treatments, but extended chilling increased carcass weight. Carrying-transport decreased *P. major* yield and increased blood appearance (?), whereas extended chilling-deboning also decreased P. major yield but had no effect on blood appearance. Reduced lightness (L) and increased redness (a) occurred with carrying-transport while extended chilling-deboning increased redness and yellowness (b). *P. minor* yield was not affected by carrying-transport but myopathy increased, whereas extending chilling-deboning reduced yield without altering myopathy.

Résumé

Des poulets ont été abattus après un stress "normal" ou sans avoir été transportés. Les carcasses ont été refroidies dans la glace fondante pendant 6 ou 30 h puis les filets ont été collectés. Le rendement carcasse et le gras abdominal ne dépendaient pas du traitement avant l'abattage, mais l'allongement du refroidissement augmentait le poids de carcasse. Le transport avant abattage réduisait le rendement en muscle *Pectoralis major* et augmentait la fréquence des points de sang dans ce muscles., alors que l'allongement du refroidissement réduisait également le rendement en *P. major* mais n'affectait pas l'apparence de ce muscle. Le transport réduisait la luminance (L*) et augmentait l'indice de rouge des *P. major* alors que l'allongement du refroidissement augmentait les indices de rouge et de jaune (b*). Le rendement en muscle *P. minor* n'était pas affecté par le transport mais ce dernier augmentait la présence de sang. Un refroidissement prolongé diminuait le rendement en *P. minor*.

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Introduction

Yield and quality of broiler breast meat are known to suffer from the stresses associated with bird transfer from farm to processing plant. Breast meat decreases more than any other tissue with transport (Moran and Bilgili, 1995). Vibration with movement has been shown to elevate body temperature and predispose a decrease in the pH of breast meat (Warris et al., 1997). Such excitement appears to accentuate redness of the final product that is not related to blood (Ngoka and Froning, 1982). Hemorrhaging into meat is multifaceted with physical handling being the primary culprit (Kranen et al., 2000). Handling that encourages wing movement is also known to facilitate the occurrence of deep pectoral myopathy (Siller et al., 1978).

Processing and associate *post mortem* changes may also alter breast meat yield and quality. Tenderness of breast meat substantially improves through the first several hours post mortem (Lyon et al.1992; Young, 1997); however, color and cooking loss are not perceptibly altered (Papinaho et al., 1996). Wing activity from post-stun electrical stimulation to enhance post mortem glycolysis and tenderness also decreases muscle pH at the time, but differences ultimately equalize as the duration of chilling is extended (Gault et al., 2000). Mechanized catching and improved environment during transport can substantially decrease the stress perceived by broilers (Duncan et al., 1986; Nicol and Scott, 1990). Present experimentation examined the significance of the omission of carrying after catching and motion of transport on breast meat from heavy broilers, and whether modifications of these effects would occur after processing from an extended chill and duration before its removal from the carcass.

Materials and Methods

A total of 1600 male chicks (Ross x Hubbard HiY) obtained from a commercial hatchery were randomly distributed to 32 floor pens (4.2 sq m /pen) having pine shaving litter. All birds received common corn soybean meal feeds to 8 weeks of age (21.8% CP & 3.03 kcal ME/g, 0-17 days; 20.0 & 3.10, 17-38; 19.2 & 3.16, 38-45; and 18.4 & 3.17, 45-56, respectively). At 56 days of age, birds were either carried approximately 25 m by one leg in groups of three from pen to coops on a truck or cooped directly at the pen and coop carried to the truck. Birds that had been carried were subsequently transported in coops for one hour then held stationary until processing, whereas those cooped the pen were directly transferred for holding at the processing plant. A total of 12 hours from catching until slaughter occurred with both preslaughter treatments.

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On-line processing involved a 9-minute kill line followed by a 7-minute evisceration line until chilling. Warm carcasses from each preslaughter treatment were divided and subsequently static submersion slush-ice chilled either 6 or 30 hours. Immediate to chilling, fat was removed from the abdominal cavity followed by carcass deboning by experienced commercial personnel using stationary cones. Blood contaminating the *P. major* and *P. minor* indicating myopathy were ascertained concurrent with removal of breast meats from the carcass. Light reflectance was measured at the center on the skin side of all *P. major* muscles at 48 hours post-mortem using the Hunter scale with a Minolta hand-held spectrophotometer. Analysis of variance based on a factorial arrangement of the two preslaughter treatments and two chilling durations were the basis of statistical evaluation.

Results and Discussion

The feeding regimen and environment provided to broiler males used in present experimentation led to a favorable final performance at 56 days of age (live weight= 3665 g; F/G= 1.96; total mortality= 10.4%). Omitting both carrying and actual transportation during the 12 hours preslaughter was intended to simulate optimization of advantages using mechanical catching and improved conditions possible from optimal truck design; thereby, enabling a defined comparison to stresses existing under present circumstances. Moran and Bilgili (1995) imposed 6 hours of transport to broilers and observed a significant loss in body weight that was particularly apparent with breast meat compared to those kept stationary. Imposing carrying together with one hour of transportation failed to adversely affect loss in either abdominal fat or yield of the resultant carcasses in present experimentation (Table 1), but a decrease in the *P. major* was perceptible (Table 2). Extending submersion chilling from 6 to 30 hours increased carcass weight; however, subsequent removal of the *P. major* indicated a substantial loss in yield that could largely be attributed to the associated improved tenderness and adherence of portions to the skeletal frame (Table 1). The *P. major* from broilers that had received carrying-transport exhibited additional blood attributable to vascular rupture that was not altered by extended chilling-deboning. Measurements conducted 48 hours *post mortem* indicated a reduction in lightness (L) when additional preslaughter stress had been applied as well as increased redness (a). Areas of blood contamination and reflectance measurements differed; thus, the influence of one on the other seemed unlikely. Although extended chilling-deboning did not alter lightness, redness and yellowness (b) increased independent of preslaughter stress.

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The *P. minor* responded to carrying-transport and chilling in a different manner than the *P. major* (Table3). Carrying-transport did not perceptibly alter yield, whereas the improved tenderness arising from the delay from 6 to 30 hours *post mortem* led to a loss in its recovery from the carcass. The incidence of deep pectoral myopathy was extensive with these broilers, and an increased incidence with carry-transport was apparent. Wing flapping facilitates an ischemia that may affect all or part of the muscle (Martindale et al., 1979). Reddening appears with affected areas prior to the traditional gangrenous appearance. Its reduced incidence with extended chilling-delayed deboning occurs independently and probably relates to muscle changes that decrease detection. Overall results indicate that minimizing preslaughter stresses to improve bird welfare will benefit both breast muscles as will extended *post mortem* duration, but the basis of theses changes are different and independent of one another.

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Table 1. Influence of preslaughter stress and extended static slush-ice chilling on carcass yield and abdominal fat from 8 week old broiler males[1]

Carry & Transport	Chill Hrs	Abdominal Fat[2] g Wt.	Abdominal Fat[2] % Carcass	Carcass w/o Abd. Fat[3] g Wt.	Carcass w/o Abd. Fat[3] % Live
Nil	6	70	2.77	2505	67.4
	30	71	2.69	2556	67.9
Yes	6	69	2.69	2503	67.4
	30	71	2.72	2522	67.8
SEM		1.2	0.063	16.1	1.11
Stress		NS	NS	NS	NS
Chill		NS	NS	**	NS
Interaction		NS	NS	NS	NS

[1]Values are the least-square means of 10 carcass from 32 total pens.
[2]Fat removed from the abdominal cavity expressed on an absolute basis and as a percentage of the whole carcass.
[3]Carcass without neck after static slush-ice chilling for the respective durations and removal of abdominal fat expressed on an absolute basis and relative to the full-fed live weight.
NS, P>.05; **, P<.01

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Table 2. Influence of preslaughter stress and extended chill-debone on yield and quality of the *P. major* from 8 week old broiler males[1]

Carry & Transport	Chill Hrs	Yield[2] g Wt.	% Carc.	Blood[3] % Strained	Hunter Reflectance[4] L	a	b
Nil	6	588	23.5	4.3	48.9	0.03	4.26
	30	574	22.5	4.9	49.1	0.25	4.44
Yes	6	583	23.3	5.6	48.2	0.20	4.10
	30	559	22.1	5.5	48.4	0.46	4.41
SEM			0.11	0.09	0.57	0.144	
		12.8					0.211
Stress		*	*	*	*	**	NS
Debone		***	***	NS	NS	***	***
Interaction		NS	NS	NS	NS	NS	NS

[1]Values are the least-square means of measurements performed on 10 carcasses from a total of 32 pens.
[2]Absolute amount and its percentage of the chilled carcass without abdominal fat.
[3]Relative incidence of visually obvious blood.
[4]Hunter scale values taken at the center of the surface from the skin side.
NS, P>.05; *, P<.05; **, P<.01; ***, P<.001

Table 3. Influence of preslaughter stress and extended chill-debone on yield and quality of the *P. minor* from 8 week old broiler males[1]

Carry & Transport	Chill Hrs	Yield[2] g Wt.	% Carcass	Myopathy[3] %
Nil	6	133	5.3	5.2
	30	130	5.1	4.7
Yes	6	132	5.3	9.7
	30	129	5.1	9.0
SEM		2.3	0.06	0.07
Stress		NS	NS	***
Debone		*	*	*
Interaction		NS	NS	NS

[1]Values are the least-square means of 10 carcasses from a total of 32 pens
[2]Absolute amount and its percentage of the chilled carcass without abdominal fat.
[3]Relative incidence of myopathy.
NS, P>.05; *, P<.05; ***, P<.001

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THE INFLUENCE OF MECHANICAL *vs* ELECTRICAL STUNNING ON BLOOD LOSS AND PRODUCT DOWNGRADING IN FORCE-FED GEESE

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Keywords: geese, force-feeding, electrical stunning, mechanical stunning

Abstract

The experiment compared the effects of four treatments (three stunning methods and no stunning) on the rate of blood loss and on the incidence of appearance defects on fatty liver and muscles in force-fed geese (n= 124) and ganders (n= 127). The stunning methods were : electrical stunning in a water bath with a sinusoidal AC of 50 Hz, 50 mA for 4 s, 'head-only' stunning with a sinusoidal AC (50 Hz, 200 mA, 4 s) and mechanical stunning using a penetrative captive bolt. Captive bolt stunning gave the highest extent of blood loss after 180 s (similar to non-stunned, birds) in both geese and ganders, whereas head-only stunning gave the lowest one. Water-bath stunning resulted in more petechial haemorrhages in breast muscle and fatty liver (ganders only) than other methods, whereas the best result was obtained with both no stunned and captive bolt stunned birds.

Résumé

L'effet de quatre traitements avant l'abattage (trois méthodes d'étourdissement et saignée sans étourdissement) sur l'amplitude de la saignée et les défauts de présentation des foies gras et des muscles a été étudié chez des oies mâles (n= 127) et femelles (n= 124). L'étourdissement consistait en l'une des techniques suivantes : électronarcose en bain électrifié (courant alternatif sinusoïdal de 50 Hz, 50 mA, 4 s), électronarcose « tête seulement » (courant alternatif sinusoïdal de 50 Hz, 200mA, 4 s), pistolet à tige perforante. La meilleure saignée était obtenue après étourdissement au pistolet (similaire aux non étourdis), et la plus mauvaise après électronarcose « tête seulement ». L'incidence des pétéchies dans les aiguillettes et sur les foies gras (seulement chez les mâles) était la plus élevée après électronarcose en bain électrifié,

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alors que les animaux non étourdis et ceux étourdis au pistolet présentaient les meilleurs résultats.

Introduction

The production of overfed geese for fatty liver consumption has increased in France during the past years (+15 % from 1999 to 2000) and has reached about 1 million overfed animals. Downgrading of fatty liver, due to gross appearance defects, is a crucial commercial issue since this product has a high added value. The main appearance defects reported by processing plants are the overall redness of the liver, which is thought to be highly dependent upon the efficiency of blood loss, the presence of haematoma and petechial hemorrhages and the red color of the lobe tips. Though the etiology of these defects has not been studied in details, several empiric observations, carried out under commercial conditions, suggest a major role of the stunning process (Leprettre, unpublished observations). In France, overfed geese are usually stunned individually in a water bath using an AC 50 Hz sine wave current of varying intensity and/or tension. With this system, the current goes through the whole body and induces strong muscle contractions. Carcass quality defects such as broken bones and petechial haemorrhages in muscles and other tissues may result from these contractions (see for instance Fernandez *et al*., 2003, for force-fed geese). In the present work, two alternative stunning methods were compared to the water bath in term of extent of blood loss and carcass and fatty liver quality.

Material and Methods

A total of 124 geese and 127 ganders (*Anser anser*) from the French Landes grey breed were used. The animals were raised at the Station of Goose Breeding (Coulaures, France) until the age of 12 weeks, following standardized practices (Leprettre *et al.*, 1997). The animals were then overfed in collective pens (10-12 animals/pen; pen size 3 x 1 m) during 19 days. Overfeeding was achieved by the distribution of a soaked-corn mixture (grain-flour; 42-58) in four meals per day, using an hydraulic machine. The quantity distributed was adapted to the ingestion capacity of each animal.

The animals were slaughtered in the experimental abattoir of the Station of Goose Breeding (Coulaures, France), over one day. The last meal was given 8 h before slaughter. The geese were suspended head downwards from a shackle and individually

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stunned, according to the treatments described below. A group was slaughtered without stunning.

- water bath stunning with a sinusoidal 50 Hz AC (50 mA / bird) for 4 s,
- head-only stunning with a sinusoidal 50 Hz AC (200 mA) for 4 s,
- mechanical stunning with a spring-handled penetrative bolt. The instrument, designed for rabbit stunning, was applied in the center of the top of the skull.

Birds were unrestrained and therefore allowed to convulse. At 10 s after the end of the stun, a bilateral section of the carotid arteries and the jugular veins was performed. Bleeding was allowed for 3 min before entering the scalding tank.

Blood was collected in a plastic bag suspended around the neck of the animal. The amount of blood loss was measured by weighing the blood and expressed as % of live weight. The carcasses were then eviscerated (25-30 min *post mortem*). The fatty liver was collected and the appearance defects usually reported under commercial conditions were subjectively scored. These include haematoma, petechial hemorrhages and red color of the lobe tips. *Petechiae* were recognized as small pin-point like bloodspots at the surface of the liver, originating from capillarie damages. At 24 h *post mortem*, the carcasses were cut and breast muscles were harvested. The incidences of petechial hemorrhages on *pectoralis major* and *pectoralis minor* muscles, red wing tips and fracture of the proximal end of the humerus bone (*caput humeri*) were recorded as presence or absence.

For numerical data, analyses of variance were performed using the GLM (General Linear Model) procedure of SAS. The model included the main effect of sex, stunning method and the interaction. When variance analysis revealed a significant effect, differences between groups were tested using LSD multiple range test. Subjective score data were treated by non parametric analysis of variance, using the 'NPAR1WAY' procedure of SAS. Where appropriated, means were compared using the Wilcoxon non parametric test for paired-mean comparison.

Results and discussion

Stunning method affected significantly the extent of blood loss over the first three minutes after the blood vessels cut (Figure 1).

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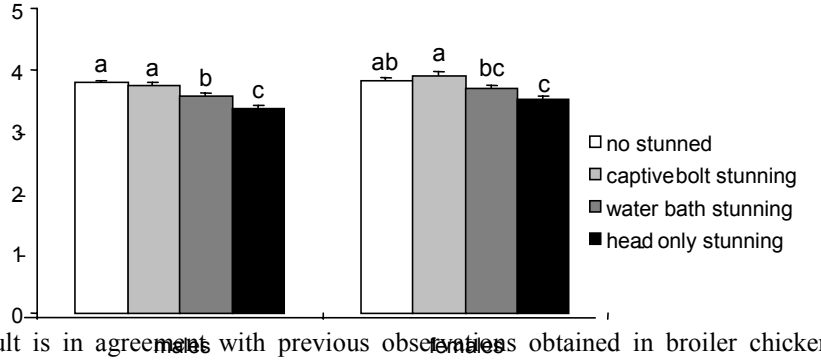
Figure 1 : Effect of stunning method on blood loss (% of live weight) in ganders and geese (vertical bars show the standard error of the mean)



This result is in agreement with previous observations obtained in broiler chicken where captive bolt stunning has been shown to induce a higher blood loss after neck cutting than head-only electrical stunning (Kotula & Helbacka, 1966a). In addition, as in the present case, Kotula & Helbacka also reported a similar blood loss over 3 min in non stunned and captive bolt stunned birds. However, our result do not support the observations of Lambooij *et al.* (1999) and Göksoy *et al.* (1999) who found a lower blood loss in broiler chicken killed after mechanical stunning, compared to electrical water-bath stunning. In poultry, and more specifically in turkey, it has been shown that birds killed by the stun lost less blood than those who were still alive at the point of neck cutting (Mouchonière *et al.*, 1999). In the present case, the differences in blood loss between the stunning methods cannot be explained by differences in the incidence of dead animals at the point of neck cutting since none of these methods kills the birds. An effect of stunning method on blood distribution in the body, and subsequently on blood loss, is more likely, as early evidenced by Kotula & Helbacka (1966b) who showed that differences in blood loss between various stunning techniques was due to differences in blood retention in the neck, heart, viscera, head, spleen and kidney of broiler chickens.

The incidences of red wing tips, petechial haemorrhages on muscle *Pectoralis major* and fractures of the proximal end of the humerus bone (*caput humeri*) were either very low or nil and did not depend upon stunning methods. Therefore, the corresponding results are not shown in Figure 2. As shown in Figure 2, the incidence of petechial haemorrhages on the fatty liver of ganders was significantly higher in water-bath stunned birds than in the other three groups. In geese however, the incidence of petechial haemorrhages on fatty liver did not depend on stunning method. Whereas the *Pectoralis major* muscle did not show any petechial haemorrhages, the incidence of this defect was noticeable in *Pectoralis minor*, and it strongly depended on stunning method. In ganders, non stunned birds did not show this defect, its incidence was very

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low after captive bolt stunning, whereas the highest rate was observed in water-bath stunned animals, head-only stunning giving intermediate results. A similar situation was observed in geese with the exception that the incidence of this defect after captive-bolt stunning was not significantly different from that of non stunned animals. Overall, these results confirm previous observations obtained from other poultry species where electrical stunning in a water-bath gives usually the highest incidence of petechial haemorrhages in muscles, as compared to other stunning methods (see for instance Schreurs *et al.*, 1999; Lambooij *et al.*, 1999). In addition, our results show that captive bolt stunning gave the lowest incidence of petechial haemorrhages in muscle, among the various stunning methods. This beneficial effect of captive bolt stunning, compared to electrical stunning, has been previously demonstrated in other poultry species (Göksoy *et al.*, 1999; Lambooij *et al.*, 1999).

Conclusions

The present data show the beneficial effect of captive bolt stunning in force-fed geese as compared to electrical stunning, either head-only or in a water-bath. This effect concern the extent of blood loss and the incidence of petechial haemorrhages in muscles and, for males only, in fatty liver. Further research is needed to confirm this effect under commercial slaughter conditions.

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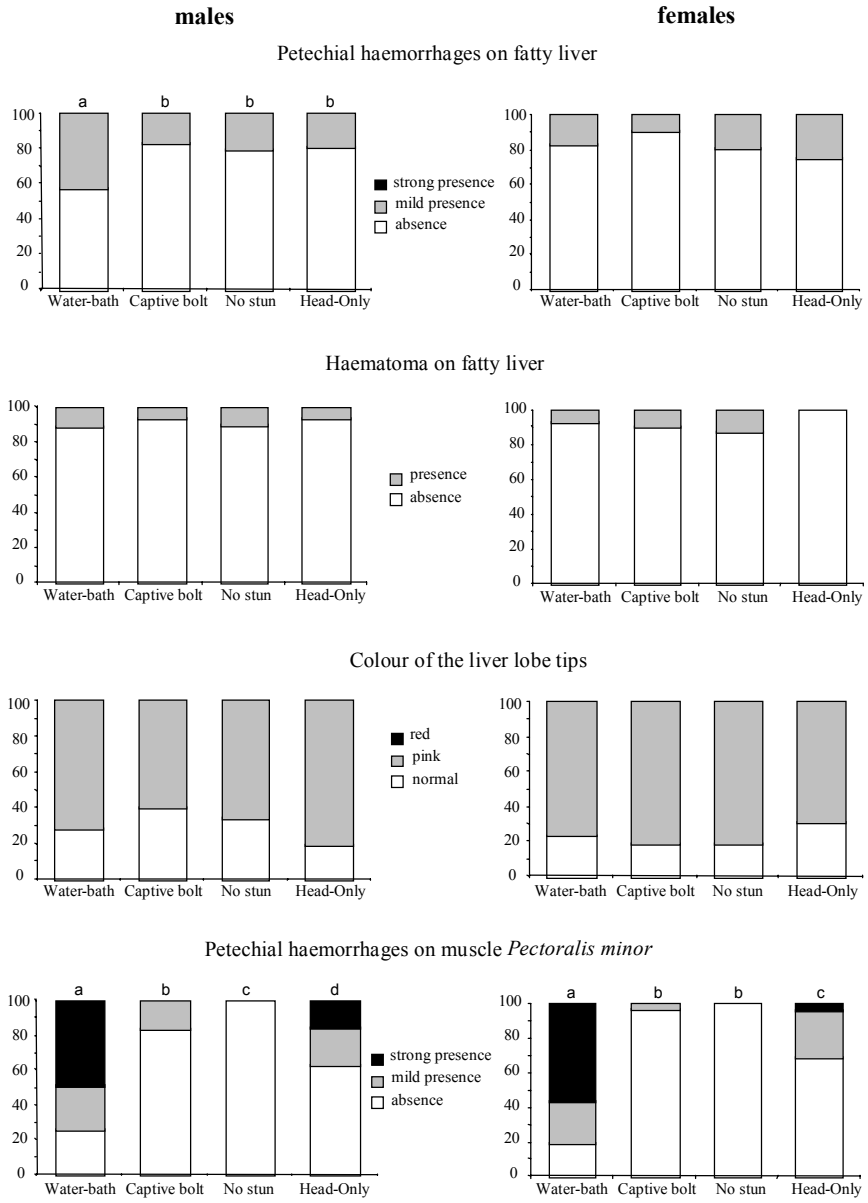
Figure 2 – Incidence of various appearance defects according to stunning method (data are expressed as %)



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CONTROLLED ATMOSPHERE STUNNING OF CHICKENS WITH A CARBON DIOXIDE-OXYGEN MIX IS PREFERABLE TO A CARBON DIOXIDE-ARGON MIX

A. M. L. Coenen, J. A. C. Lankhaar and E. L. J. M. Van Luijtelaar

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Keywords: Anaesthesia, euthanasia, stunning, slaughtering, poultry, broiler chickens, turkeys, carbon dioxide, argon, oxygen, electroencephalogram, heart rate, behaviour

Abstract

Controlled atmosphere stunning (CAS) of poultry as an alternative for electrical stunning, was investigated. Chickens were brought in a controlled atmosphere and two different gaseous stunning methods were tested. The electrical brain activity, heart rate and behaviour were recorded to follow the induction of unconsciousness and death. The first method was based on creating a hypercapnic anoxic condition using carbon dioxide and argon, while the second method comprised of two phases. In the first phase animals were anaesthetised with a relative high oxygen and a low carbon dioxide level, while in the second phase the birds were introduced in a mix of a high carbon dioxide and a low oxygen level, leading to a soon death. Birds reached unconsciousness slower and died significantly later in the mix of oxygen with carbon dioxide. However, in the carbon dioxide and argon mix the animals showed strong signs of agitation and distress. These signs occurred in a period in which consciousness could still not be fully excluded. It is concluded that, although the chickens are faster unconscious and also die sooner in the argon/carbon dioxide condition, the oxygen/carbon dioxide condition is to be preferred. A milder death is preferred to a quicker but more distressing death. Similar results were obtained for turkeys.

Résumé

L'anesthésie en atmosphère contrôlée (controlled atmosphere stunning = CAS) de volailles a été comparée à l'électronarcose. Des poulets ont été introduits dans une atmosphère contrôlée et deux méthodes différentes d'anesthésie par le gaz ont été

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testées. L'activité électrique cérébrale, le rythme cardiaque et le comportement ont été enregistrés après l'induction de l'inconscience et de la mort. La première méthode est basée sur la création d'une condition hypercapnique anoxique à l'aide de dioxyde de carbone et d'argon, alors que la seconde méthode comprenait deux phases. Dans la première phase, les volailles étaient anesthésiées dans une atmosphère à niveaux relativement élevé d'oxygène et bas d'acide carbonique. Dans la seconde phase, les volailles étaient introduites dans un mélange à niveau élevé de dioxyde de carbone et à niveau bas d'oxygène, ce qui menait rapidement à la mort. Les volailles perdaient conscience plus lentement et étaient mortes considérablement plus tard dans le mélange d'oxygène et d'acide carbonique. Toutefois, dans le mélange d'acide carbonique et d'argon les volailles ont montré de forts signes d'agitation et de détresse. Ces signes se sont produits dans une période où la conscience ne pouvait pas encore être complètement exclue. Il est conclu que, bien que les poulets perdent conscience et meurent plus rapidement dans le mélange d'argon et de dioxyde de carbone, le mélange oxygène/acide carbonique est à préférer. Une mort plus douce est préférable à une mort plus rapide mais plus stressante. Des résultats semblables ont été obtenus pour les dindes.

Introduction

Presently, electrical stunning of poultry is the most common method used in commercial slaughter practice. For reasons of animal welfare high currents are used, which lead to severe muscle contractions, resulting in meat quality problems. An alternative for electrical stunning is controlled atmosphere stunning, eliminating the negative effects on animal welfare and meat quality. In slaughter practice, only gases that naturally occur in air, such as carbon dioxide, nitrogen, oxygen and argon, are applied. Carbon dioxide is an agent often used for stunning of small animals and is recommended as an efficient way to induce a painless and humane death (AVMA, 1993). Despite the fact that carbon dioxide induces an unconscious state quickly, considerable agitation and distress is caused in the short period before unconsciousness appears. As an acidic gas, carbon dioxide causes pungent feelings leading to pain and distress (Forslid et al., 1986). Also in use is carbon dioxide in combination with inert gases such as nitrogen and argon. According to Raj and Whittington (1995), chickens can be killed in argon without inducing strong respiratory discomfort before the loss of consciousness. Nevertheless, behavioural signs of agitation are reported in applying argon stunning (Lambooij et al., 1999). Alternatively, carbon dioxide in combination with oxygen is in use. Supplementation of oxygen minimises the signs of agitation and asphyxia, but is critical because it

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affects the time to death (Coenen et al., 1995). Thus, stunning with carbon dioxide may be optimised by the addition of oxygen.
The central position adopted here is that a method for stunning poultry before slaughter is acceptable when it results in minimal signs of agitation and distress in the period that the electroencephalogram (EEG) is still either normal or shows a pattern in which some degree of consciousness could not be excluded (Coenen et al., 1995). This implies that the first stage of the stunning method should be the induction of an adequate anaesthesia, followed by a second stage in which the birds reach a state of irreversible anaesthesia until slaughter is accomplished. Here, the effects on chickens in the period preceding slaughter of two different gaseous stunning methodologies are described. The two methods were tested with EEG, heart rate, and behavioural recordings, to follow the induction of unconsciousness and death. The first method is based on creating a hypercapnic anoxic condition, using carbon dioxide and argon. The second method is a two-phase system, in which the birds are first anaesthetised in an atmosphere with a high level of oxygen and low level of carbon dioxide, while subsequently the birds are introduced in an atmosphere with a high level of carbon dioxide and a low level of oxygen quickly leading to the death of the animals.

Materials and Methods

Eight chickens of six weeks old were provided with an EEG electrode set and with two clamps, fixed in the skin, for heart rate recording (Coenen et al., 2002). Chickens were placed in a cage for 5 minutes, for heart rate and baseline EEG measurement (1 - 70 Hz) and for assessment of their behaviour. Subsequently, the chickens were placed in a cabin filled with 30% carbon dioxide, 60% argon in air with 8% nitrogen and less than 2% oxygen. This is the ‘argon condition’, in which 4 chickens were placed. Also 4 chickens were placed in a mix with 30% oxygen, 40% carbon dioxide and 30% nitrogen for one minute, followed by 80% carbon dioxide, 5% oxygen and 15% nitrogen. This is the ‘oxygen condition’. EEG, heart rate and behaviour were recorded for 10 minutes. The EEG recordings were divided into phase 1, with a normal EEG pattern, phase 2, with a reduction in the general amplitude, and phase 3, with an iso-electric EEG. The heart rate was measured at several times. Distinguished in the behaviour of the chickens were the occurrence of purposeful movements, gasps and intense muscle contractions. Finally, the latency till immobility was measured. Differences in behaviour were subjected to a one-factor analysis of variance with subsequent post-hoc tests carried out according to Tukey ($p<0.05$), with an independent sample t test.

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Results and Discussion

In Figure 1, an EEG of a chicken in the two conditions is shown. Phase 1 (a normal EEG pattern), was similar in length in both conditions (5-10 sec.). However, there were differences in the duration of phase 2 (the time till unconsciousness) and hence, in the onset of phase 3 (the iso-electric phase) ($p < 0.05$). The mean ± SD latency time to unconsciousness was 22.6 ± 11.7 sec. for the argon condition, and 62.3 ± 25.6 sec. for the oxygen condition. The exact point of death is difficult to establish. Here, a chicken was considered to be dead when the EEG was iso-electric, in combination with a very low heart rate of 180 heart beats per minute or less (normal about 300 beats). There was a difference ($p <0.01$) in the time of death. The mean time of death was 135.3 ± 35.7 sec. for the argon condition and 248.7 ± 4.9 sec. for the oxygen condition. In phase 1 gasps were more frequent in the argon condition; in phase 2 gasps occurred in both conditions, while in phase 3 no or only a few gasps occurred. In both conditions non-purposeful and intense muscle contractions did not occur in phase 1. In phase 2 these phenomena were only present in the argon condition in all birds. Although none of the birds was motionless in phase 1, in both conditions some chickens lay still in phase 2. There were no differences for immobility: 38.3 ± 3.8 sec. (argon condition) and 31.1 ± 9.5 sec. (oxygen condition). In Figure 2 heart rate changes in the two conditions are shown. Heart rate decreased in the argon condition; slightly during the first 50 sec., and quickly until 150 sec. After 500 sec. heart rate could no longer be measured. In the oxygen condition, heart rate initially decreased moderately during the first 100 sec. Then, a relatively fast decrease was observed and the heart stopped after 500 sec. The major changes in heart rate coincided with the period that the EEG was iso-electric.

The time that it takes for an animal to die is important, but the way it dies is perhaps even more important. Earlier mentioned was that chickens are considered to be unconscious when the EEG shows an iso-electric pattern (phase 3). When a chicken is unconscious, it is less important what happens. It is thus relevant what is happening during phase 1 and phase 2 of the EEG. In the oxygen condition, chickens are longer conscious and it takes longer before they die, than in the argon condition. However, in the argon condition there are much more intense gasps in EEG phase 1, implying that chickens experience severe distress, being fully conscious. Also strong and intense muscle contractions were seen in phase 2 in all the chickens of the argon condition, while no such contractions were present in phase 2 of the oxygen condition. In phase 2, consciousness cannot fully be excluded. This means that chickens may experience strong muscle contractions in the argon condition, which is undoubtedly distressing. Finally, it was striking that 3 out of 4 chickens were vomiting in the argon condition. Vomiting is caused by stress and hypoxia.

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In a preliminary study four turkeys were also placed in the oxygen condition. The procedures were identical to those used for the chickens and identical anaesthesia methods were applied. A representative profile of a baseline EEG together with the changes in the pattern is shown in Figure 3. In general, there is a correspondence with the changes in the broiler EEG. When the movement artefacts associated with placing the bird in the atmosphere ceased, the amplitude decrease had already started (phase 2). This passed into an iso-electric EEG (phase 3). Hence, it seems that turkeys respond in the same way to the oxygen condition as chickens.

It is concluded that chickens reach unconsciousness slower and die significantly later in the oxygen/carbon dioxide condition as judged by the EEG, heart rate and behaviour. However, in the carbon dioxide/argon condition the birds show strong signs of agitation and distress. The disturbed behaviour and the signs of distress occur in a period that consciousness cannot fully be excluded. Thus, it is proposed that, although the chickens lose consciousness sooner and die sooner in the carbon dioxide/argon condition, the oxygen/carbon dioxide condition is preferred. A milder death (defined as an iso-electric EEG together with a low heart rate), which takes somewhat longer, is preferred to a quicker but more distressing death.

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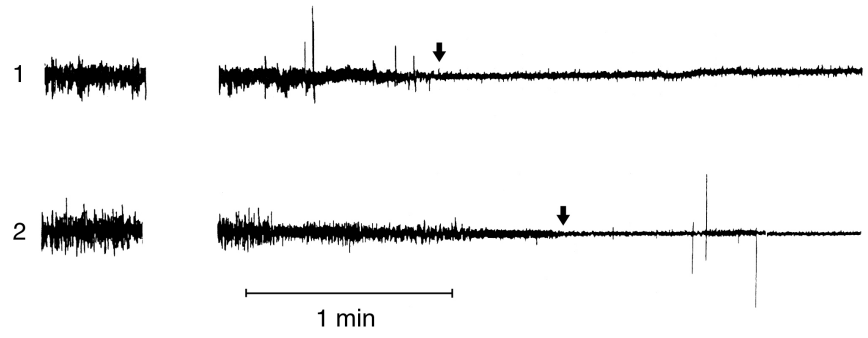

Figure 1. The EEG of a chicken in the carbon dioxide/argon condition (1), and in the oxygen/carbon dioxide condition (2). First, 30 sec of the baseline is shown, followed by a recording, starting when the chickens are placed in the gas condition. Arrows mark an iso-electric EEG.

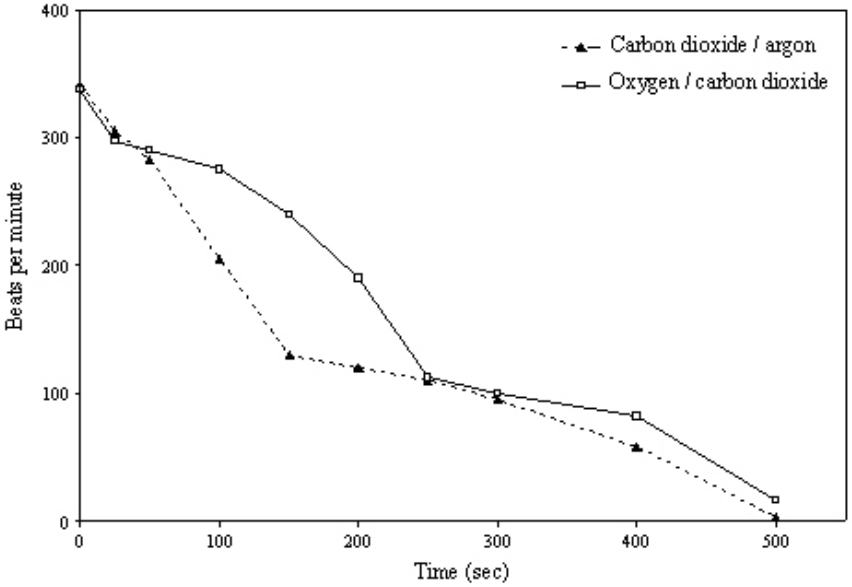

Figure 2. Changes in heart rate of broiler chicken in the two conditions. The main changes occur when the EEG is already iso-electric.

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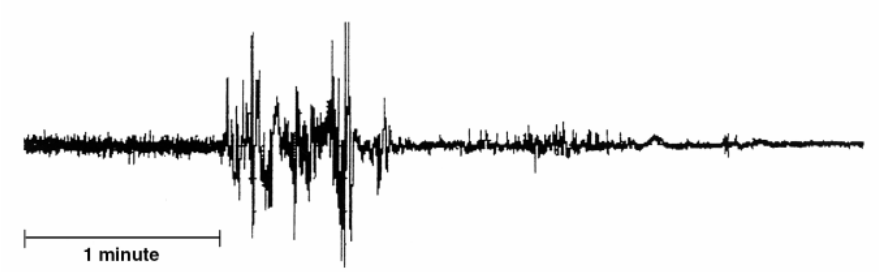

Figure 3. The EEG of a turkey in the oxygen/carbon dioxide condition. In the middle of the period movement artefacts, due to placing the animal in the cabin, can be seen. This occurs just before the EEG reaches iso-electricity.

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RAPID RIGOR: THE WAY TO ACCELERATE IN-LINE POULTRY PROCESSING

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Keywords : electrical stimulation, tenderness, rigor mortis, in-line processing

Abstract

Tenderness is a primary quality attribute of meat. Deboning muscles, which have not developed full rigor mortis, will cause them to contract and hence to shorten, because there is still some energy left in the tissue. The result is tough meat. Broiler pectoral muscles can take 6 hours to develop full rigor mortis. In-line processing and portioning of poultry is a full continuous process without buffering. It therefore allows deboning at 3 hr post mortem. However, at 3 hr post mortem many carcasses will not have developed full rigor mortis. Therefore, there is a risk of tough meat. Electrical stimulation of plucked carcasses, accelerates the post mortem energy metabolism of the muscles. As a result, the energy will be depleted within 3 hr post mortem. As compared to non-stimulated muscles, deboning of stimulated ones at this point in time results in considerably lower shear values. It is concluded that electrical stimulation of plucked carcasses accelerates rigor mortis development. It therefore, allows deboning of breast muscle at 3 hr post mortem, and hence in-line processing and portioning, without detrimental effects on meat tenderness.

Resumé

La tendreté est une caractéristique majeure de la viande. Le désossage avant l'entrée en rigor induit une contraction musculaire importante et, par conséquent, un raccourcissement du muscle, le muscle n'ayant épuisé complètement ses réserves Il en résulte une viande plutôt ferme. L'installation de la rigor dans les muscles pectoraux de poulet se réalise en 6 heures en moyenne.. Dans le cas d'un abattage des poulets par un processus continu et rapide, le dessosage s'effectue en général trois heures après la mort. Or à ce stade, beaucoup de carcasses n'ont pas encore développé la rigidité cadavérique complète. Par conséquent, le risque d'obtenir une viande ferme voire dure est important. Une stimulation électrique des carcasses après plumaison épuiserait les

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réserves énergétiques des muscles en moins de trois heures après la mort. Comparé aux muscles non-stimulés, lesmuscles stimulés ont des valeurs de force decisaillement considérablement moins élevées. Donc, la stimulation électrique des carcasses, en accélérant le développement de rigidité cadavérique permet un désossage de muscles pectoraux trois heures après la mort sans altérer la tendreté de la viande.

Introduction

Tenderness is a primary quality attribute of meat. Tenderness of broiler breast meat basically depends on the moment of deboning in relation to the development of full rigor mortis and the proteolytical breakdown of myofibrillar proteins, resulting in loss of structural integrity. Upon pre-rigor boning, muscles will be able to shorten. As a result, bonds between the myofibrillar structural components will be either increased or strengthened. The result is tough meat (Papa and Fletcher, 1988; Papa and Lyon, 1989).

Shortening and hence toughening of meat is caused by contraction of muscle tissue in which the energy has not been entirely depleted at deboning. Residual energy is present as adenosine tri-phosphate (ATP). After slaughter ATP synthesis predominantly depends on anaerobic glycolysis, due to cessation of the oxygen supply. As a result, lactate will start to accumulate, lowering the pH of the muscle. At a pH of about 5.8, glycolysis in the chicken breast muscle will stop. No more ATP will be formed. At the moment the ATP is depleted, the muscle will go into full rigor mortis. Since several definitions of rigor mortis are used in literature, it should be noted that full rigor mortis is defined as the moment the muscles have been depleted of energy and become inextensible (Goll et al., 1970). It may take 6 hours for chicken pectoral muscle to reach full rigor mortis. Muscles in a state of full rigor mortis will no longer be able to shorten at deboning. As a result, the meat will no longer toughen.

Methods to monitor the post mortem process are to determine the progress of the glycolyis, by measuring the lactate formation, pH decline, and ATP depletion. A simple method to assess ATP depletion is to determine the R-value (Honikel and Fischer, 1977).

Nowadays, in-line processing of poultry from the moment of killing to portioning is a full continuous process without buffering. It allows deboning at 3 hr post mortem. As a result, one day of shelf life is gained. Additional benefits are in-line grading, control and direction of the further processing, as well as adequate tracking and tracing. However, at 3 hr post mortem many carcasses will not have developed full rigor mortis. Therefore, there is a risk of tough meat.

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Electrical stimulation of the carcass at bleeding can accelerate rigor development considerably, however not always consistently (Alvarado and Sams, 2000, Uijttenboogaart, 2001). To avoid stimulation of live animals and problems with feather removal, electrical stimulation can be performed following bleeding, scalding and plucking. The objective of the study was to investigate whether electrical stimulation applied after bleeding and defeathering can accelerate rigor development in such a way that in-line processing and portioning can be applied without a risk of tough meat.

Materials and methods

Chickens from two commercial broiler parental lines (Nutreco, Boxmeer, The Netherlands) were slaughtered at an age of 40 days. They were electrically stunned, exsanguinated for 3 min, scalded and plucked. Subsequently, half of the carcasses were electrically stimulated (ES group), half was not (NES group). Stimulation was performed by applying electrical pulses for 5 minutes at a moderate voltage (220 V, 50Hz). The carcasses were manually eviscerated and subsequently chilled in a cold store, until deboning. Deboning was performed at 0.5, 1.5, 3, 6 and 10 hrs post mortem (40 carcasses per group per deboning time).
After deboning, one of the muscles from the superficial pectoral muscle pair was used for pH, ATP and lactate measurement as well as for determination of the R-value (Savenije et al., 2002). The other was weighed, left to age for 72 hrs and weighed again to determine the loss of tissue fluid during storage. Subsequently it was heated for texture analysis using a Warner-Bratzler shear cell (Froning and Uijttenboogaart, 1988). Sarcomere length was determined by laser diffraction pattern analysis (Pospiech and Honikel, 1987).

Results and discussion

In non-stimulated carcasses lactate continued to accumulate up to 6 hours post mortem (figure 1). As a consequence, the pH did not reach its ultimate level of 5.8 until that time. These results indicated that glycolysis and hence energy generation kept on going during this entire period. Indeed, ATP level and R-value showed that energy depletion did not occur until 6 hrs post mortem (figure 2). It was concluded that in non-stimulated carcasses, full rigor mortis took about 6 hrs to develop.
Electrical stimulation accelerated lactate accumulation and, as a result, pH decline. It also stimulated energy depletion, as indicated by the lower ATP concentrations and R-values being measured (figure 2). In the non-stimulated group, lactate as well as pH

reached their ultimate level as early as 3 hours post mortem. By this time all the energy had been depleted and hence full rigor mortis had been developed as well.
Shear values showed that deboning muscles pre-rigor, caused toughening of the meat (figure 3). At three hours post mortem, shear values were high in the non-stimulated group, whereas they were already low in the stimulated one, which had already developed full rigor mortis by that time. Surprisingly, there was no significant correlation between sarcomere length and shear values (r^2=0.05). The average sarcomere length varied between 1.82 and 1.88 µm. Therefore, it is not certain that sarcomere shorting is the mechanism by which meat toughening due to pre-rigor boning is caused.
No significant differences in tissue fluid lost from the meat during storage were found. It indicates that acceleration of the pH decline by electrical stimulation did not negatively affect the water holding capacity of the meat.
It can be concluded, that electrical stimulation of plucked carcasses accelerates rigor mortis development. It, therefore, allows deboning of breast muscle at 3 hr post mortem, without detrimental effects on meat tenderness. Electrical stimulation, incorporated in the slaughter line, therefore makes in-line processing and portioning of broiler chickens possible without the risk of tough meat.

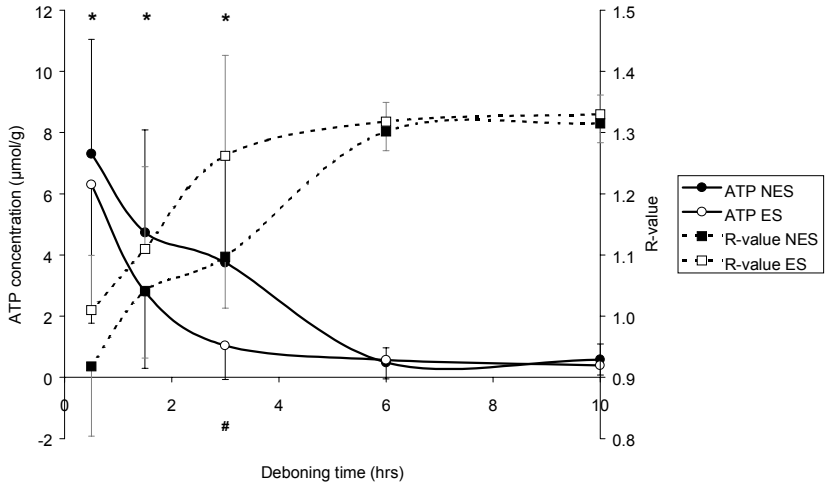

Figure 1. pH and lactate concentration measured at various deboning times in the superficial pectoral muscle (sd indicated). Significant difference (p<0.05) in pH (#) or lactate concentration (*) between the ES (stimulated) and NES (non-stimulated) group.

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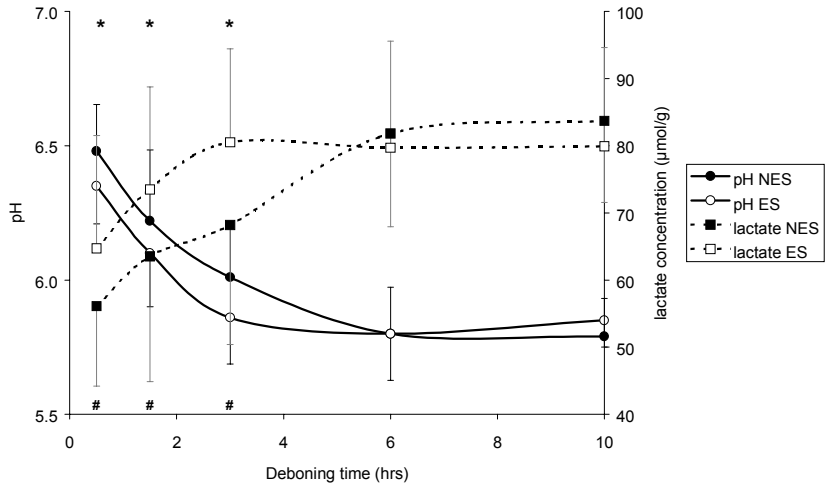

Figure 2. ATP concentration and R-value at various deboning times in the superficial pectoral muscle (sd indicated). Significant difference (p<0.05) in ATP concentration (#) or R-value (*) between the ES (stimulated) and NES (non-stimulated) group.

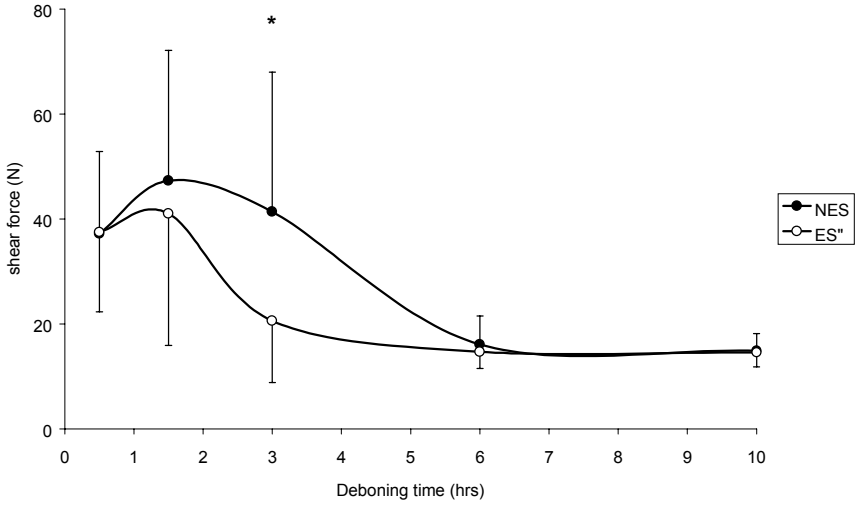

Figure 3. Shear values at various deboning times in the superficial pectoral muscle (sd indicated). *:Significant difference (p<0.05) between the ES (stimulated) and NES (non stimulated) group.

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CONTENTS

CORRELATION OF *P. MAJOR* L* FROM A BROILER POPULATION AT 8 WEEKS WITH LIVE WEIGHTS AND PROCESSING YIELDS, THEN FREEZE-THAW AND COOKING CHANGES USING POPULATION EXTREMES

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Keywords: breast meat, cooking loss, freeze-thaw, light reflectance, *P. major.*

Abstract

500 broiler males were individually weighed at 21, 42 and 56 days, processed under common terms, and deboned 24 hours later. CIE light reflectance was performed on the *P. major* from each carcass 24 hours after deboning, then the entire population was held frozen (-20ºC). L* values were positively correlated with live weights at 21 and 42 days of age and gain from 21 to 42 days. L* was positively correlated with the relative carcass yield from live and *P. major* from carcass but not their absolute weights. Negative correlations existed for percentages of abdominal fat and *P. minors* obtained from the carcass. Sub-samples representing the *P. majors* having the highest (68.4), lowest (56.8), and median (62.9) L* values were thawed (3 days at 4ºC) and oven cooked (163ºC to 80C internal). L* values increased from raw to cooked and differences between classes were reduced. Absolute fresh raw weights of representatives from each class were equivalent; however, the percentage weight losses associated with thawing plus cooking progressively increased with L*. Moisture percentage as well as reduction in length and width after cooking were similar among samples, whereas thickness increase after cooking was minimal with the group having the highest L* values when raw.

Résumé

L'expérience a été réalisée sur 500 poulets mâles pesés individuellement à 21, 42 et 56 jours d'âges puis abattus et désossés 24 h plus tard. La réflectance du P. major (L*) a été mesurée 24h plus tard avant congélation de la viande. Les valeurs de L* étaient positivement corrélées aux poids vifs à 21 et 42 jours, au gain entre ces deux âges, aux rendements en carcasse et en P. major et négativement corrélées avec les pourcentages

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de gras abdominal et de P. minor. Des échantillons de P. majors issus de 3 classes de L*, haute (68,4), basse (56,8) ou moyenne (62,9) ont ensuite été décongelés et cuits au four. Les valeurs de L* augmentaient avec la cuisson et les différences entre classes s'en trouvaient réduites. La perte au cours de la séquence décongélation-cuisson augmentait avec le L*. L'humidité relative et la réduction en longueur et largeur après cuisson étaient similaires entre groupes alors que l'augmentation d'épaisseur suite à la cuisson était minimale dans le groupe présentant les valeurs de L* les plus élevées.

Introduction

Pale, soft, and exudative (PSE)-like fillets have become an increasing problem with broilers grown to heavy weight. PSE meat is known to have a low pH, reduced water holding capacity (WHC), and additional cooking loss (Allen et al., 1998; Owens et al., 2000; Woefel et al., 2002). Lightness (L*) values obtained from light reflectance have been shown to be negatively correlated with WHC (Barbut, 1993; McCurdy et al., 1996; Allen et al., 1998; Fletcher, 1999; Le Bihan-Duval et al., 1999) and better relate its functionality than pH (Woefel et al., 2002). No information exists on the relationship of L* values either to fillet losses after freezing and thawing or possible relationship to the broiler's preceding growth and carcass characteristics. In present experimentation, the L* values of fillets obtained from a normal population of broiler males at 56 days of age was correlated with their body weights at intervals throughout live production and chilled carcasses after processing, then freeze-thaw and cooking alterations of representatives from the median and extremes were measured.

Materials & methods

A total of 500 day-old male chicks originating from a Ross × Ross 308 breeder flock received common feed and management from placement to 56 days of age. Birds were individually weight at 21, 42 and 56 days of age then "on-line" processed together and static slush-ice chilled for 4 h. Fillets (*Pectoralis major*) and tenders (*Pectoralis minor*) were removed from each chilled carcass 24 h postmortem. 24 h later, light reflectance (CIELAB-L*) was measured on fillets from the right side of each carcass (illuminant D65 and standard 10º observer). All readings were located at the center of the muscle surface that had been adjacent to the skin. Fillets were individually bagged and frozen (-20ºC), then those having the highest, lowest and median L* values corresponding to the total population (33 fillets per category) were weighed and thawed (3 days at 4ºC). Each thawed fillet was subsequently weighed, its maximum

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length, width and thickness determined, and L* value measured at the same location. Thawed fillets were cooked in a preheated (163ºC) electric oven to a fixed internal temperature (80ºC) as measured by indwelling thermocouples. Cooked fillets were allowed to cool for 45 min before weighing, measurement of dimensions, and L* determination on an exposed surface. Cooked fillets were lyophilized to estimate their free water content. One and the same person conducted respective measurements at each stage of experimentation.

Data regarding weight and light reflectance measurements on raw, thaw and cooked samples, and dimensions in thaw and cooked samples were analyzed as repeated measures by using the MIXED procedure of SAS (2001). Thawing, cooking and total losses as well as change in dimensions were evaluated by analysis of variance in a completely randomized design. Pearson's Correlation Coefficients and probabilities were generated using the correlation procedures of SAS (2001).

Results and discussion

L* is an easy procedure to perform and detect fillets having a PSE-like problem. Escalation of PSE-like problems with age suggests that the preceding rate and nature of growth may predispose its incidence. Present experimentation measured individuals in an entire population of broiler males at intervals throughout live production and characteristics of their carcasses after processing then correlated each factor with their respective fillet L* value. L* value at 48h postmortem was correlated ($P \leq 0.05$) with live weight at 21 ($r = 0.10$) and 42 ($r = 0.11$) days of age as well as interim weight gain from 21 to 42 days ($r = 0.09$) but not with live weight at 56 d or weight gain from 42-56 days. L* was also correlated ($P \leq 0.05$) with percentage yield of chilled carcass from its full-fed live weight ($r = 0.09$) and relative amounts of total fillets removed from the carcass ($r = 0.11$); however, negative correlations existed with absolute ($r = -0.12$) and relative amounts ($r = -0.14$) of abdominal fat as well as the proportion of tenders ($r = -0.11$).

A normal distribution of L* values existed with the entire population of fillets that were common in strain source, sex, terms of production, and conditions of processing. A total of 33 sub-samples representing the median (mean = 62.9; range 62.8-63.1) and extreme low (mean = 56.8; range 51.7-58.3) and high (mean = 68.4; range 66.9-73.2) values led to fillets with maximal differences in L* values uncomplicated by background. These fillets were used to examine the significance of L* to changes associated with freezing, thawing and cooking.

L* of fillets after freezing and thawing decreased with values that were previous extremely high and increased when extremely low (Table 1). Cooking led to a further increase in L* with all fillets from the thawed state which was more extensive with the

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samples that had extremely low than extremely high values. Little information exist on fillet L* values beyond the fresh state. Lyon et al. (1976) did not observe differences in Hunter L values of fillets between samples kept fresh and those frozen 6 days then thawed at 2 ºC overnight. In the present study, sub-samples at 48 hours post mortem having L* at the median did not change from fresh with freezing and thawing. Overall research generally supports that L* values of fillets increase through the first 24 hours postmortem with little change thereafter; however, samples involved random representatives from a population (McCurdy et al., 1996; Qiao et al., 2002; Mollete et al., 2003). Cooking is known to greatly increase fillet L* values (Yang and Chen, 1993; Lyon et al., 1998; Fletcher et al., 2000). The net effect of freezing, thawing, and cooking was to reduce difference in L* values between extremes within the whole population, which agrees with the results of Fletcher et al. (2000).

Weights of fillets after removal from the carcass were similar, regardless of their L* value, and subsequent losses with thawing from the frozen state (*ca.* 10%) and cooking (*ca.* 26%) were extensive (Table 2). However, the influence of their L* value was small and not statistically significant until both were combined. As L* of the initial raw fillet increased so also did the percentage total loss ($r = 0.27$, $P \leq 0.01$). Correlations between L* and losses during storage and cooking of breast fillets have been well established (Allen et al., 1998; Le Bihan-Duval et al., 1999; Owens et al., 2000; Polidori et al., 2000; Woefel et al., 2002). Lyophilization measured fillet water content, and no differences could be detected (grand mean = 64.5%, $P > 0.05$) nor was time needed to achieve 80ºC altered (grand mean = 38.9 min, $P > 0.05$), regardless of L* value.

However, our results and those mentioned above clearly establish that there is a direct relationship between lightness of meat and its WHC (measured by the water losses during freeze-thawing and cooking). In our experiment, total losses were higher for the fillets lighter in color. Similarly, Allen et al. (1998) and Woefel et al. (2002) found that both drip and cooking losses of broiler breast meat were higher for those fillets that were pale than for those classified as dark. In turkeys, Barbut (1993) found a 9% difference in cooking loss between breast fillets having very light (53.6) or very dark (41.1) L* values 24h postmortem.

The fillet represents a muscle entirely of fast glycolytic fibers oriented in one direction, and the extensive losses from cooking are expected to alter its dimensions (Table 3). Cooking significantly reduced length (51 mm, 24.5%) and width (17 mm, 15.2%) independent of L*, whereas thickness increased in a manner that was minimal with fillets that had the highest L*. Presumably, increased preparation losses with high L* fillets is compensated by reduced thickness given equivalent moisture content. Lyon et al. (1997) observed a 22% reduction in length and 15% reduction in width of broiler breast meat that had been immersed in water at 85 ºC for 30 min while Papa and Lyon (1989) noted a reduction of 24.3% in length after oven cooking. Both reports did not mention change in thickness.

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Overall results from the present study indicate that "on-line" measurement of L* can be used to segregate fillets and optimize their subsequent use. Correlations with early growth suggest some relief from PSE-like problems may occur through flock nutrition and management at this time.

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Table 1. Variation in L* among fillets having extreme values from raw to thawed and cooked state.

L-category	Raw-Thaw	Thaw-Cooked	Overall
Low	1.46[a]	25.23[a]	26.70[a]
Medium	-0.09[b]	22.42[b]	22.33[b]
High	-2.42[c]	19.54[c]	17.11[c]
SEM	0.467	0.489	0.308
P	***	***	***

[1]Each value is represented by measurements on 33 fillets from each L-category.
[a, b, c]: Values within columns without common superscript are significant different (P≤ 0.05). NS, P > 0.10; ***, P ≤ 0.001.

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Table 2. Weight and loss after thawing and cooking of breast fillets having extreme L-values at processing.[1]

L-category	Fillet weight (g)			Weight loss (%)[2]		
	Raw	Thawed	Cooked	Thaw	Cook	Total
Low	320	289	216	9.7	25.1	32.4[b]
Medium	325	292	215	10.2	26.2	33.7[ab]
High	332	296	216	10.8	26.9	34.9[a]
SEM	7.4	7.4	3.4	0.23	0.38	0.34
P	NS	NS	NS	NS	NS	*

[1]Each value is represented by measurements on 33 fillets from each L-category. [2] Thaw = Thawing loss from raw state; Cook = Cooking loss from thawed state; Total = Thawing plus cooking losses from raw state. [a, b]: Values within a column without common superscript are significant different ($P \leq 0.05$). NS, $P>0.10$; *, $P \leq 0.05$.

Table 3. Change in physical dimensions from thawed to cooked state of fillets having extreme L-values at processing.[1]

	Thawed dimensions (mm)			Cooked dimensions (mm)			Δ %		
L-category	Length	Width	Thick	Length	Width	Thick	Length	Width	Thick
Low	206	112	24	155	96	31	-24.6	-14.0	+27.6[a]
Medium	206	113	23	156	96	30	-24.1	-15.0	+28.2[a]
High	202	112	24	152	94	29	-24.9	-16.5	+20.5[b]
SEM	1.1	0.7	0.3	0.9	0.7	0.3	0.48	0.58	1.35
P	NS	NS	NS	NS	NS	NS	NS	NS	*

[1]Each value is represented by measurements on 33 fillets from each L-category. [a, b]: Values within a column without common superscript are significant different ($P \leq 0.05$). NS, $P>0.10$; *, $P \leq 0.05$.

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OBJECTIVE CRITERIA TO DIFFERENTIATE CARCASS AND MEAT QUALITY OF LABEL AND STANDARD GUINEA FOWLS

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Keywords: guinea fowl, meat, carcass, muscle, quality

Abstract

This paper is a synthesis of a research programme concerning control of carcass and meat quality of guinea fowls. By comparison with standard guinea fowls, skin colour of label carcass was darker and redder which confer them a "game bird" connotation required by the consumer. The "ready to cook" carcass yield of label guinea fowls was lower but their meat yield was equivalent to those of standard birds while subcutaneous fatty depot was higher. Regarding technological quality, label guinea fowls produced breasts with higher rate of post-mortem pH decrease, lower cooking loss and higher resistance to compression test. With regard sensorial qualities, breasts of label guinea fowls were judged firmer and their thighs had a more pronounced flavour.

Résumé

Cette revue est la synthèse d'un programme de recherche concernant le contrôle de la qualité de la carcasse et de la viande de pintades. Par comparaison avec les pintades standards, la couleur de la peau des carcasses labels est plus foncée et plus rouge ce qui leur confère une connotation gibier recherchée par le consommateur. Le rendement en carcasse « prête à cuire » des pintades labels est plus faible mais le rendement en viande est équivalent à celui des pintades standards et le dépôt de gras sous-cutané est plus important. Concernant la qualité technologique, les pintades labels produisent des filets présentant une chute de pH post-mortem plus rapide, des pertes à la cuisson inférieures et une résistance plus élevée à un test de compression. Sur le plan de la qualité sensorielle, les filets des pintades labels sont jugés plus fermes et leurs cuisses présentent une flaveur plus prononcée.

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Introduction

In France, production of guinea fowls was 64,000 tons in 2001, representing 2.5 % of total poultry meat production. There are three major production systems: standard (65 %), certified (5 %) and « label rouge » production (30 %). Relationships between quality of poultry meat and label production system have mainly been studied for broiler chickens. It was clearly shown that the major factors influencing carcass composition and meat quality were genotype in relation with age (Touraille et al., 1981a; 1981b; Culioli et al., 1990). Composition of diets, rearing density and access to a pasture had lesser effects (Ricard, 1988). Rearing conditions for « label rouge » production have been strictly defined. For guinea fowl, their effects on growing performances have been already studied (Blum and Leclercq, 1979). However, the influence of genotype and rearing conditions on carcass and meat quality in guinea fowl were still unknown. Three trials were achieved to determine objective criteria to differentiate carcass and meat of label and standard guinea fowls and which factors (genotype-slaughter age and/or rearing system including rearing density, specific diets and access to a pasture) explained the observed differences (Baéza et al., 2002a).

Materials and methods

For each trial, we reared 390 male label and 390 male standard guinea fowls (Galor Company) in a closed poultry house. They were allocated to 4 groups as shown in table 1. The slaughter age and genotype were linked for the trials 1 (realised during winter) and 2 (realised during summer). Only the season was different between trials 1 and 2. For the third trial (realised during spring) the slaughter age was linked to the rearing system to determine more precisely the effect of genotype on the different measured criteria. Inside the poultry house, the same temperature and lighting programmes recommended by the Galor Company were applied for all the trials. The main characteristics of diets (starter, grower and finisher) are shown in table 2. After 6 or 8 weeks of age (spring and summer or winter period), birds reared under label conditions had access to a pasture (2 m^2/guinea fowl).
Growth rate and feed conversion ratio were calculated by individually weighing of animals and measuring food consumption at different ages. Ninety guinea fowls per group were slaughtered at 77 and 98 days for standard and label genotype respectively (trials 1 and 2) or for standard and label rearing conditions (trial 3). The colour of the skin was measured on the breast part under the wing of live birds (one day before their slaughter) and their carcass (one day after their slaughter) with a Miniscan TM colorimeter (Hunterlab, CIELAB system: L* = lightness, a* = redness, b* =

yellowness). The « ready to cook » (RTC) and cut yields (breasts, thighs with shanks, wings) were measured on 50 guinea fowls per group (Marché, 1995). The carcass fatness was estimated by weighing the abdominal fat and measuring the wing membrane thickness with a micrometer. Breast muscles of 20 guinea fowls per group were excised and weighed. On the left breast, the pH at 15 min and 24 h post-mortem (2 g of muscle ground in 18 ml of 5 mM sodium iodo-acetate) was measured. A breast sample was frozen and stored at -20°C for determining chemical composition. Water (AOAC, 1996), mineral (AOAC, 1996), protein (Kjeldhal; AOAC, 1996) and lipid (Folch et al., 1957) contents were measured. We measured drip loss on the right breast muscle after 3 day's storage at + 4°C, colour (1 and 3 days after slaughter) with the Miniscan TM colorimeter, cooking loss (10 min at 85°C) and resistance to compression test at 20 % (K20) or 80 % (K80) of sample height (Culioli et al., 1990). Sensory analysis of breast and thigh meat was performed on 20 roasted carcasses per group by 12 trained persons (Baéza et al., 2001).
For the sensory analysis, we compared the means with a Tukey test. The other data were compared with a variance analysis (Newman- Keuls test).

Results and discussion

The following results are only a synthesis of the comparison between groups SS (standard guinea fowls reared under standard conditions) and group LL (label guinea fowl reared under label conditions) because for the three trials, the animals of these groups, were always reared under the same conditions and slaughtered at the same ages (table 1). For these groups, only the season and therefore the outside conditions were different between each trial. The results of the other groups (SL and LS) will be used to explain the observed differences. The whole data for each trial were previously detailed by Baéza et al. (2002a).

1. Growth and carcass composition

At 28, 56 and 76 days of age, standard guinea fowls were significantly heavier than label guinea fowls: + 6, + 7 and + 9 % respectively (table 3). This difference was induced both by the effects of genotype and rearing conditions. Standard guinea fowls had a lower feed conversion ratio than label guinea fowls: 2.83 vs 3.05. With the access to a pasture (climate and exercise), label guinea fowls had higher needs. Their diets were less concentrated (table 2) and their rearing period was longer. Feed conversion ratio of label guinea fowls was higher during winter than during summer (3.24 vs 2.96) because low temperatures induced higher feed consumption (Baéza et al., 2002b).

The skin was thicker in label guinea fowls than in standard guinea fowls suggesting a higher subcutaneous fat deposition: 101 $\pm$ 14 µm vs 96 $\pm$ 13 µm. The skin thickness

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depended on genotype and slaughter age (Baéza et al., 2002a). Before and after slaughter, the skin of label guinea fowls was darker and presented higher intensity of red and yellow than the skin of standard guinea fowls (table 4). The genotype had moderate influence on the skin colour. This character was mostly influenced by the rearing conditions and particularly the exposition to sunlight. Then, during summer this difference was more marked. Label guinea fowls slaughtered at 98 days' old were significantly heavier (+ 13 %) than standard guinea fowls which were slaughtered at 77 days' old (table 5). The same trends were observed for the weight of RTC and the different cut parts (table 5). Standard guinea fowls had a higher RTC yield. Meat yields were slightly or not influenced by genotype and rearing conditions (table 5). Baéza et al. (2002b) showed that meat yields of label guinea fowls slaughtered at 87, 94 and 101 days were equivalent. In spite of a longer rearing period for the label guinea fowls the percentage of abdominal fat was equivalent to that of standard guinea fowls. For this character, the influence of age was balanced by the access to outside and a lower energy concentration in diets.

2. Technological quality of breast meat

The rate of decrease in pH was slower in breast of standard guinea fowls than in breast of label guinea fowls (table 6). This was already described in standard chicken compared to label chicken (Culioli et al., 1990) suggesting different activities of glycolytic metabolism in these genotypes. Ultimate pH was not influenced by genotype neither rearing conditions. Drip loss after 24 h storage at +4°C was 2.56 % and it was not influenced by genotype neither rearing conditions (table 6). Cooking loss was higher for standard guinea fowls (table 6) and the difference with label guinea fowls was induced by genotype effect (Baéza et al. 2002a). Genotype and rearing conditions had moderate effect on the colour of breast meat (Baéza et al., 2002a). The breast of label guinea fowls had a higher resistance to compression test than breast of standard guinea fowls: 12.14 ± 4.17 N/cm^2 vs 11.11 ± 4.80 N/cm^2 and 102.71 ± 39.81 N/cm^2 vs 91.46 ± 34.02 N/cm^2 for K20 and K80 respectively.

3. Chemical composition of breast meat

Lipid content of breast meat was lower for label guinea fowls (table 6) and the difference with standard guinea fowls was induced by rearing conditions. Genotype and rearing conditions had moderate effect on water, protein and mineral contents of breast muscle.

4. Sensorial analysis of breast and thigh meat

Differences between standard and label birds were low. The tenderness of breast was higher and the flavour of thighs was lower for standard guinea fowls and these characteristics were mainly determined by genotype (Baéza et al., 2002).

From the whole results, we can describe specific effect of genotype, rearing conditions, age and season on growth, carcass traits and meat quality of label and standard guinea fowls. **The label genotype** had lower growth rate and thicker skin. In their breast muscles, the rate of decrease in post-mortem pH was higher but cooking

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loss was lower. Their breasts were less tender and their thighs had a flavour more marked. **The label rearing conditions** induced lower growth rate and higher feed conversion ratio. The percentage of abdominal fat was lower. The skin was thinner, darker with higher intensity of red and yellow. Breast muscles had higher resistance to compression test and lower lipid content. **The increasing age** at slaughter induced higher feed conversion ratio, live and cut part weights, carcass fatness and lower meat tenderness. **The season** influenced feed conversion ratio (higher during winter), meat and carcass fatness (higher contents during summer), skin colour (darker during summer), rate of decrease in post-mortem pH, drip loss and cooking loss in breast muscle (higher during summer).

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Table 1: Rearing conditions of the four groups of guinea fowls for the trials 1, 2 and 3 (slaughter age in brackets)

Group	Genotype	Slaughter age (days)	Diet	Density (animals/m^2)	Access to a pasture
SS	Standard	77 (77)	Standard	17	No
LL	Label	98 (98)	Label	13	Yes
SL	Standard	77 (98)	Label	13	Yes
LS	Label	98 (77)	Standard	17	No

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Table 2: Main characteristics of diets (ME = metabolisable energy, CP = crude protein)

Periods (weeks)	**0-4**		**4-8**		**8-slaughter**	
Diets	**Label**	**standard**	**label**	**standard**	**label**	**standard**
ME kcal/kg	2850	2950	2950	3050	3050	3150
CP g/kg	220	230	195	205	165	180

Table 3: Body weight (BW in g) of standard and label guinea fowls (n = 195 per group for each trial). Means and standard errors were calculated with the results of three trials.

Groups	**BW at D28**	**BW at D56**	**BW at D76**	**BW at D97**
Standard	535 ± 55 a	1374 ± 105 a	1822 ± 137 a	
Label	505 ± 49 b	1288 ± 94 b	1673 ± 114 b	2041 ± 146

a, b : significant difference between groups for one given parameter with $P < 0.05$

Table 4: Skin colour characteristics of standard and label guinea fowls, before and after slaughter (n = 50 per group for each trial). Means and standard errors were calculated with the results of three trials.

Criteria		**Standard**	**Label**
Before slaughter	L*	59.40 ± 2.68 a	50.65 ± 2.49 b
	a*	1.26 ± 0.65 b	2.69 ± 0.52 a
	b*	- 0.06 ± 1.37 b	1.21 ± 0.99 a
After slaughter	L*	53.74 ± 2.77 a	45.07 ± 4.03 b
	a*	1.89 ± 0.67 b	3.39 ± 0.68 a
	b*	2.26 ± 1.57 b	3.45 ± 1.16 a

a, b : significant difference between groups for one given parameter with $P < 0.05$

Table 5: « Ready to cook » (RTC) and cut yields of label and standard guinea fowls (n = 50 per group for each trial). Means and standard errors were calculated with the results of three trials.

Criteria	**Standard**	**Label**
Body weight (g)	1788 ± 135 b	2020 ± 129 a
RTC yield (%)	71 ± 1 a	69 ± 1 b
Abdominal fat (%)	2.1 ± 0.6	1.9 ± 0.5
Thighs-shanks (%)	25.0 ± 0.8	24.8 ± 0.7
Breasts (%)	16.4 ± 0.9	16.7 ± 0.8
Wings (%)	7.5 ± 0.4	7.1 ± 0.4

Yields were calculated by comparison with body weight at slaughter age.
a, b : significant difference between groups for one given parameter with $P < 0.05$

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Table 6: Post-mortem pH fall, drip loss after a 3 day storage at + 4°C, cooking loss (10 min. at 85°C) and chemical composition of breast from standard and label guinea fowls (n = 20 per group for each trial). Means and standard errors were calculated with the results of three trials.

Criteria	Standard	Label
pH 15 min	6.24 ± 0.11 a	6.12 ± 0.12 b
pH 24 h	5.66 ± 0.09	5.68 ± 0.09
Drip loss after 4°C storage (%)	2.61 ± 0.72	2.50 ± 0.73
Cooking loss (%)	9.89 ± 1.56 a	8.51 ± 1.66 b
% Proteins	24.65 ± 0.56	24.48 ± 0.45
% Lipids	1.42 ± 0.38 a	1.15 ± 0.34 b
% Water	73.74 ± 0.53	74.03 ± 0.56
% Minerals	1.12 ± 0.09	1.08 ± 0.09

a, b : significant difference between groups for one given parameter with $P < 0.05$

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STATUS OF OSSIFICATION OF BREAST BONE AS A CRITERION FOR DISCRIMINATING BETWEEN YOUNG AND ADULT PEKIN HYBRIDS

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Keywords : Pekin duck, carcass, breast bone, ossification, age

Abstract

According to EU marketing regulations for poultry meat ducks have to be marketed either as young ducks (with flexible breast bone processus) or as ducks (with rigid breast bone processus). As the type of duck hybrids has changed markedly due to intensive selection during past years the question arises whether the course of ossification of the breast bone has been changed as well. In the study different characteristics of the breast bone were determined in drakes and ducks between weeks 3 and 20 of age: weight, metric dimensions and chemical composition. The results indicate that deposition of inorganic matter in breast bone is finished at the end of week 7, the caudal end of breast bone is ossified in week 18 and dry matter content is increasing till week 20.

Résumé

Selon les règlements de L´UE concernant la commercialisation de la viande de canard il y a une catégorie jeune canard (bréchet partiellement cartilagineux) et canard (bréchet ossifié). Les souches utilisées ayant été modifiées par une sélection intensive pendant de nombreuses années, nous avons cherché à savoir si l'ossification a été également changée. Dans la présente étude nous avons étudié les caractéristiques suivantes du bréchet de mâles observés entre les âges de 3 et 20 semaines: Poids corporel, dimensions et composition chimique. Les données démontrent que le dépôt de la matière minérale dans le bréchet est terminée à la fin de la 7ème semaine, la partie caudale du bréchet est ossifiée à partir de la 18ème semaine et la teneur en matière sèche augmente jusqu'à la 20ème semaine d'âge.

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Introduction

According to EU order (EWG) 1906/90 Pekin ducks (*Anas platyrhynchos dom.*) have to be marketed either as young ducks (with flexible breast bone processus) or as ducks (with rigid breast bone processus) depending on the age of ducks. In intensive Pekin duck production duration of fattening has been reduced to 46 days, meanwhile, and will be reduced even further. Only few literature is available dealing with the course of the ossification process in Pekin ducks. Clayton and Draper (1970) used an x-ray apparatus, Swatland (1979) metric measures of the breast bone, Sager *et al.* (1986, 1987, 1988) body dimensions and Kelley *et al.* (1993) ash content of tibia. But, all these determinations were not directly related to the ossification process in the breast bone. Lilburn stated in 1994 that older measures may not be valid for today's Pekin duck breeds as the development of the skeleton has been heavily accelerated due to the enormous breeding progress. Based on the current knowledge in Pekin ducks the objectives for the present research have been set to define accurate indicators for the status of ossification of the breast bone and to fix an age where the ossification process is obviously finished.

Material and Methods

In total 360 male and female Pekin ducks of the breed Stolle-Seddin (Altfriedland, Germany) were used. Ducks were kept in groups of 10 male and 10 female ducks in pens of 12 m² in a windowless poultry house between day of live 4 and 20 weeks. Ducks were fed commercial starter (weeks 1 and 2; 21 % CP, 11.8 MJ ME/kg, 0.99 % Ca, 0.71 % total P), grower (weeks 3 to 8; 17 % CP, 12.4 MJ ME/kg, 0.88 % Ca, 0.58 % total P) and finisher (weeks 9 to 20; 16.5 % CP, 12.5 MJ ME/kg, 0.89 % Ca, 0.6 % total P) diets ad libitum. Starting on day 21 weekly birds of one pen (10 drakes and 10 ducks) were slaughtered, dissected and the breast bone was removed completely. Besides live weight, weights of carcass and breast meat were recorded. The following metric measures of the breast bone were taken (figure 1): total length, distance between processi craniolaterali, distance between trabeculae intermediae, length of trabecula intermedia, length and width of incisura medialis, height at rostrum, height of breast bone keel in the middle and height of blood supplied part of the breast bone keel. Furthermore, the contents of dry matter, ash and calcium were analyzed. Until week 7 of age the gender was not recorded. LSQ means were calculated by one way (chemical analyses) and two way (metric measurements) analysis of variance, respectively. Furthermore, simple correlations between traits were calculated. The statistic program JMP (SAS Institute) was used.

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Results and discussion

Live weight and weight of the breast muscle increased continuously till the age of 9 weeks, declined thereafter and was finished after week 11 for both sexes (table 1; combined results of sexes). From these data it can be concluded that ducks have reached their adult weight already in week 12. Distinct effects of age and sex were observed for LW and BMW.

The breast bone weight reached a maximum in week 7 of age and decreased thereafter to a steady level from week 13 to 20 (table 1). The other dimensions increased till week 9 to 11 of age, with no later obvious changes. The traits were significantly influenced by age and sex, whereas the interaction age x sex was of less importance. In general, the results are in agreement with Sager *et al.* (1986, 1987, 1988). But, due to the effects of live weight and gender the dimensions of the breast bone are no accurate indicators for the age of ducks.

Up to week 7 the breast bone consisted mainly of cartilage and was quite pale. In week 7 the breast bone turned to become darker due to the high blood and water content. After week 11 the color of the breast bone turned to more brightness and the incisura intermediae became transparent. The blood supply of the breast bone keel nearly diminished in week 20 (figure 2). The visual evaluation of the breast bone confirmed the known developmental process of this bone. In early age the bones consists mainly of cartilage. The ossification process starts with the building of blood vessels to transport calcium to the bone. The transport process is mainly finished at the end of week 7. After that, blood and water (as shown below in figure 2) are removed from the bone and replaced by air. This pneumatization is necessary in birds for weight reduction of bones (Salomon, 1993). The ossification develops from the cranial to the caudal end of the bone. The caudal end of the breast bone is fully ossified in week 16 and the trabecula intermedia in week 18.

The deposition of calcium in the breast bone is finished in week 7, whereas the dry matter content was increasing until week 20 (figure 3). This implies that the ossification process is not finished with the deposition of inorganic matter. The maximum strength of the breast bone will be achieved with the lowest physiological water content (Salomon, 1993).

Breast bone weight (BBW) was highly correlated with live weight (0.98), whereas, correlations between BBW and metric dimensions of the breast bone varied between 0.12 to 0.68. BBW was correlated to dry matter by -0.76, to ash in dry matter by 0.43 and to Ca in dry matter by -0.38. The negative correlation between BBW and dry matter is due to the weight reduction of the breast bone during aging of birds. The negative correlation between BBW and Ca in dry matter is caused by autocorrelation.

It can be concluded that none of the determined traits is suited to indicate the age of ducks during the slaughtering process with high accuracy due to the high variability in

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traits. But, the ossification process shows clear cuts: end of deposition of inorganic matter (Ca) at week 7, diminishing of cartilage by visual determination at the age of 16 weeks, end of increasing dry matter content of the breast bone at the age of week 20. As the term 'breast bone processus' is not clearly defined the age at turning to a rigid processus is not easy to fix. Nevertheless, it can be assumed that the status 'rigid' will not be reached before week 16 of live. Therefore, Pekin ducks up to an age of 16 weeks can be marketed as 'young ducks'.

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Table 1. Live weight (LW), breast meat weight (BMW), breast bone weight (BBW), length of breast bone (BBL), distance between trabeculae intermediae (DTI) and height at rostrum (HR) of ducks between weeks 3 and 20 of age (bold = reaching of maximum or plateau)

Age	LW	BMW	BBW	BBL	DTI	HR
Weeks	g	g	g	Mm	mm	mm
3	1188	27	8.1	73	45	13
4	1740	71	14.3	91	55	17
5	2176	131	21.9	109	62	21
6	2808	252	31.6	124	69	26
7	3230	374	**37.4**	134	71	33
8	3353	435	32.2	139	75	37
9	3635	497	27.8	142	**78**	38
10	3754	509	26.8	**143**	78	**39**
11	3621	493	27.3	143	78	39
12	**3939**	**532**	24.8	141	79	39
13	3923	516	24.2	141	77	39
14	3916	530	24.4	143	79	38
15	3746	526	23.6	141	77	39
16	3907	540	26.3	145	80	39
17	3789	511	24.1	143	78	38
18	3843	520	23.5	144	80	38
19	3844	497	23.2	143	78	39
20	3965	513	24.1	142	79	38
Signif.						
Age	***	***	***	***	***	***
Sex	***	***	***	***	***	***
Age x sex	**	n.s.	**	n.s.	n.s.	*

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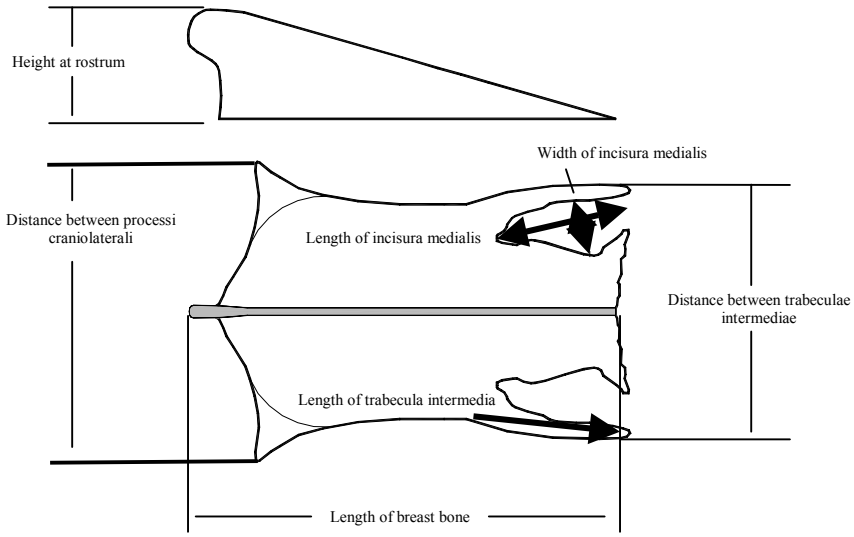

Figure 1. Definition of measurement sites of the breast bone

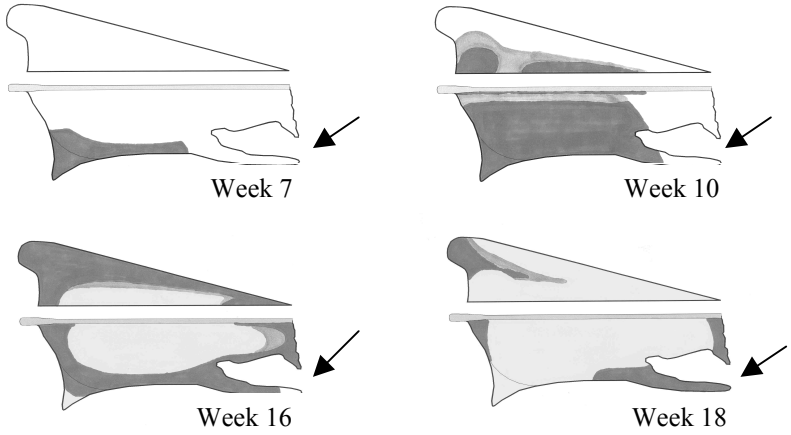

Figure 2. Process of ossification shown by the diminishing part of cartilage (arrows trabecula intermedia; transparent = cartilage; dark = supplied with blood; grey = ossified)

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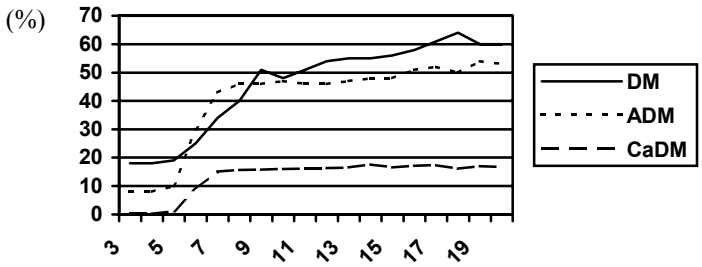

Figure 3. Change of content of dry matter (DM), ash in dry matter (ADM) and Calcium in dry matter (CaDM) in breast bones of ducks between weeks 3 and 20 of age

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THE EFFECTS OF STOCK DENSITY ON THE PREDICTION OF PERFORMANCE AND CARCASS TRAITS IN BROILER CHICKENS.

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Keywords: Broiler, Density, Growth, Carcass, Abdominal fat

Abstract

The influence of population density on the growth performance, carcass yield and abdominal fat of two hybrids chicks was studied. A total of 370 birds were housed under 10, 12, 15 and 18 birds per m^2 from 1 day-old to eight weeks of age. There was no significant hybrid difference in the considered traits. Also, there was no significant difference between densities of 10 and 12 birds per m^2, for all the recorded traits except economical result per m^2, the difference among these densities (10 and 12) and 15 and 18 were highly significant. Regression analysis showed that by increasing one bird per m^2, body weight, feed consumption, carcass yield and abdominal fat weight would decrease at the rate of -60.35, -104.79, -54.12 and -3.11 g while feed conversion ratio and economical result per m^2 would increase by 0.013 and 1.26 kg.

Résumé

L'influence de la densité en élevage sur les performances de croissance, le rendement en carcasse et le pourcentage de gras abdominal chez deux poulets hybrides a été étudiée. Un total de 370 poulets a été réparti en 4 lots différents par la densité : 10, 12, 15 et 18 poulets/m^2 de l'âge de 1 jour à 8 semaines. Aucune différence entre les deux hybrides n'a pu être mise en évidence sur l'ensemble des critères mesurés ni entre les densités de 10 et 12 poulets/m^2 à l'exception du résultat économique. Par contre les différences entre ces densités (10 et 12) et celles de 15 et 18 poulets/m^2 étaient très significatives. Une analyse de régression a montré que l'augmentation de 1 poulet/m^2 induisait une diminution du poids vif, de la consommation alimentaire, du rendement carcasse et de la quantité de gras abdominal de -60.35, -104.79, -54.12 et -3.11 g et une augmentation de l'indice de consommation et du résultat économique par m^2 de 0.013 et 1.26 kg.

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Introduction

Increasing the number of broilers in a given space is a management technique used to reduce cost associated with housing, labor and equipments. There is some evidence that excessive crowding of laying hen induced less profit (Satterlee *et al.*, 1985) and in point of broilers would lead to reduction of performance and increasing downgrading birds (Shanawany, 1988 and Cravener *et al.*, 1992). Stock density is also a public concern of some countries in the world in point of animal welfare, which is an important issue in the future of animal agriculture and research. The objectives of the present study were to compare the effect of four population densities on body weight, feed consumption and conversion, carcass weight, percentage of abdominal fat and economical result (kg per m^2 of floor).

Material and Methods

Three hundred and seventy, day-old chicks (Ross and Arian hybrid) of mixed sex were allocated in two pens according to the hybrid. At the end of the third week, the bird separated according to their sexes. Then, they were allocated in different pens to obtained densities of 10, 12, 15 and 18 birds per m^2 of floor. Each treatment had three replicates. Therefore, a sum of 48 pens was used for this experiment. Feeder and waterer were designed and adjusted to provide each bird to have access to equal water and feeder spaces in the different densities. Birds were provided *ad libitum* access to feed throughout the experiment. The starter diet was fed (2900 kcal ME/kg; 22.1% CP plus 0.47% Methionin and 1.16% Lysin) for the first two weeks. The grower diet (2900 kcal ME/kg; 21% CP plus 0.45% Methionin and 1.07% Lysin) was fed for the third to six weeks of age and the finisher diet (2900 kcal ME/kg; 19.3% CP plus 0.41% Methionin and 0.96% Lysin) was fed for the rest of the experiment. Body weight and feed intake were measured at weekly interval from four to eighth week of age. The experiment was terminated at the eighth week and all the birds processed for carcass measurements (hot carcass weight, abdominal fat weight and fat as a percentage of live weight). In addition, kg body weight production per m^2 was calculated.

Statistical analyses were performed using the General Linear Models procedure of SAS® software (SAS Institute, 1982). Factor tested in analysis-included breed (hybrid), density, sex and density by sex interactions. Means were compared using Duncan's multiple rang test (Duncan, 1995).

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Results and Discussion

There was not any significant difference ($P < 0.05$) between the two hybrids employed in the experiment for body weight at different age (Table 1). The effect of density on body weight at five, six, seven and 8^{th} week of age was highly significant (Table 1) while at 4^{th} week, it was only significant ($P < 0.05$). There was no difference between 10 and 12 bird per m^2 body weight at different ages while the differences among these densities (10 and 12 birds per m^2) and 15 and 18 birds per m^2 were significant. Results shown that this difference appeared at the end of the fourth week, just one week after the beginning of density treatment. A population density of 12 birds per m^2 resulted in the highest body weight at four and all the following weeks followed by 10, 15 and 18 birds per m^2. Some researchers believed that among environmental effects, density is one of the effective tools to reduce growth rate (Shanawany, 1988). While Cravener *et al.* (1992) reported that there was no significant difference between density of 9, 11 and 14 birds per m^2 up to 7 weeks of age. They claimed that by increasing density to 20 birds per m^2 at 7 weeks body weight would decline significantly. Buckland *et al.* (1971) also indicated that there was a significant better performance in bird gain produced at 11 birds per m^2 than those produced as a 25 birds per m^2.

There was a significant difference ($P < 0.05$) for density by sex interaction (Table 1). The report of Cravener *et al.* (1992) shown that such an interaction does not exist. Males had a highly significant higher body weight than females. Regression analysis shown that, there was a significant linear decline in body weight at four through 8 weeks of age. It means that by increasing density by one bird, it would be expected a decline of 6.43, 20.24, 34.42, 48.74 and 60.35 gr. at 4, 5, 6, 7 and 8 weeks of age. There was a significant difference in feed intake among different densities except for the densities of 10 and 12 birds per m^2. The rates of decline in intake for different densities were 8.91, 12.91, 20.74, 29.12 and 33.16 gr. per additional bird in the pen.

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Table 1. The effects of stock densities on the body weight (g) of broiler chicks[1]

Density	4 week	5 week	6 week	7 week	8 week
Overall mean	929.17	1232.58	1534.77	1839.96	2153.35
10	938.50^{A}	1286.17^{A}	1626.58^{A}	1971.92^{A}	2321.08^{A}
12	962.08^{A}	1296.75^{A}	1635.58^{A}	1983.50^{A}	2329.50^{A}
15	917.17^{B}	1209.25^{B}	1509.92^{B}	1798.08^{B}	2110.08^{B}
18	898.92^{C}	1138.17^{C}	1367.00^{C}	1606.33^{C}	1864.75^{C}
ANOVA			Probability		
Source of variation					
Breed (hybrid)	ns	ns	ns	ns	ns
Density	*	**	**	**	**
Sex	**	**	**	**	**
Density by sex	**	*	**	**	**

$^{A-C}$Means within each column with no common superscripts are significantly different ($P < .05$).
[1]Values represent x of three replicates.
ns = no significant, * = $P < 0.05$ and $P < 0.01$

As shown in Table 2, there were no significant differences for feed conversion, carcass weight, fat and fat as a percentage of body weight between 10 and 12 birds per m^2 densities. However, there was a significant difference between 10 and 12 with the rest of the densities. By increasing density, feed conversion was increased while carcass and fat weight and fat percentage were decreased. The findings of Imaeda (2000) were not supported by the present study. Imaeda (2000) shown that there were no significant differences in body weight gain and feed intake among densities of 12, 15 and 18 birds per m^2.
The interesting point was the economical result (kg of broiler produced per m^2 of pen floor). By increasing density, broiler production was increased at the rate of 1250 gr. per additional bird in the pen (Table 3). This result shows that for maximum broiler production per m^2, the higher densities are advisable. Nevertheless, one should note that by increasing kg production per m^2, carcass weight per bird would decrease which is not ideal for some market. Therefore, according to preference of size of the carcass in the market, one should make a decision for density of broiler production per m^2 of floor.

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Table 2. The effect of four varying population densities on feed conversion ratio (FCR), carcass and fat weight (g), and economical result (ER expressed as kg of broiler chicks per m^2).

Density	FCR	Carcass (g)	Fat (g)	Fat (%)	ER
Overall mean	2.15	1642.75	59.18	2.73	29.09
10	2.11^{A}	1799.83^{A}	68.33^{A}	2.95^{A}	23.21^{A}
12	2.12^{A}	1794.75^{A}	68.08^{A}	2.94^{A}	27.95^{B}
15	2.15^{B}	1586.67^{B}	55.08^{B}	2.62^{B}	31.65^{C}
18	2.22^{C}	1389.92^{C}	45.25^{C}	2.43^{C}	33.56^{D}
ANOVA		Probability			
Source of variation					
Breed (hybrid)	ns	ns	ns	ns	ns
Density	**	**	**	**	**
Sex	**	**	**	**	**
Den. by Sex	**	**	**	**	**

$^{A-D}$Means within each column with no common superscripts are significantly different ($P < .05$).
ns = no significant and ** = $P < .01$

Table 3. The expected rate of change per additional bird in the density of pen.

Traits	Rate of change (b coefficient)
Eight weeks body weight (g)	- 60.35 ± 5.05**
Carcass weight (g)	- 54.12 ± 3.76**
Feed conversion ratio	+ 0.013 ± .004**
Abdominal fat (g)	- 3.11 ± .2**
Abdominal fat (%)	- 0.07 ± .01**
Economical result (g per m2)	+ 1250 ± 70**

** = $P < .01$

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IMPROVING WELFARE CONDITIONS OF BROILER CHICKEN IN ITALY: EFFECTS ON PERFORMANCES, CARCASS AND MEAT QUALITY

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Keywords: broiler chickens, environmental conditions, carcass and meat quality

Abstract

A trial was carried out to evaluate the influence of improved housing conditions for broilers kept on litter on productivity, carcass and meat quality traits. A total of 33,000 Ross 508 female broiler chickens were divided into two groups: Welfare (WLF) and Standard (STD) and reared under different stocking density (14 *vs* 19 birds/m^2), litter (wood shaving) amount (3.5 *vs*. 2.5 kg/ m^2) and light:dark regimen (18:6 *vs*. 23:1). Birds were slaughtered at 40 or 50 d of age. WLF birds reached a higher body weight but had a slight worse feed efficiency, whereas no differences were found in carcass traits. Breast meat quality evaluation showed higher moisture content and poorer water holding capacity in WLF birds. Environmental temperature and humidity as well as litter moisture did not change between the two housing conditions.

Résumé

Un essai a été effectué pour évaluer les effets de l'amélioration des conditions environnementales des poulets élevés sur litière, sur la productivité et la qualité de la carcasse et de la viande. Un total de 33.000 poussins femelles Ross 508, ont été séparés en deux groupes : Welfare (WLF) et Standard (STD) et élevés avec des densités, des quantités de litière (copeaux) et des durées d'éclairement différentes (14 vs 19 poulets/m^2 ; 3.5 vs 2.5 kg de litière/ m^2 ; 18 vs 23 heures). Des poulets ont été abattus à 40 et 50 jours. Les poulets WLF ont atteint un poids vif supérieur mais ils avaient un indice de consommation légèrement plus élevé. Leurs filets présentaient une teneur en eau et un exsudat après cuisson supérieurs. Les caractéristiques des carcasses étaient équivalentes dans les deux groupes. Aucune différence entre les deux groupes de poulets n'a été mise en évidence pour ce qui concerne la température et l'humidité ambiante, ni pour l'humidité de la litière.

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Introduction

Animal rearing is generally carried out for commercial purposes firstly to maximise productive traits, secondarily to obtain high quality products. But birds are living and sensitive creatures and these aspects must be considered with more attention to assure them suitable welfare conditions. Animal welfare is defined by the five freedoms: freedom from thirst, hunger and malnutrition, appropriate comfort and shelter, prevention or rapid diagnosis and treatment of injury and disease; freedom to display most normal patterns of behaviour and freedom from fear (FAWC, 1993). To evaluate bird welfare, different indicators can be used such as physiological, immunological and ethological measures, health, productivity, ability to express species-specific activity including social interactions, but also the relations with housing and management play an important role in welfare conditions of birds (Broom, 1991; Rushen and De Palissé, 1992; Broom and Johnson, 1993). In 2000, the European Commission "Health & Consumer protection directorate-general" published a report on welfare of chicken in which different aspects of broiler welfare were described. In this document is also reported that in some European countries legal regulations and official recommendations have been established for broiler rearing to improve animal welfare. The authors aim to improve the environmental conditions of broiler kept in Italy and to evaluate the effects of these modified conditions on bird productivity, carcass and meat traits. In this paper are reported the results of a summer trial.

Material and Methods

In two identical poultry houses, located in the North of Italy, air conditioned, windowless and identically equipped, 33,000 Ross 508 female broiler chicks, 1 day old, were housed during summer season. The chicks were divided in two groups: welfare group (WLF) had a stocking density of 14 birds/sq m (14,000/poultry house), 3.5 kg/sq m of wood shaving as litter, a light regimen of 23 h light and 1 h dark from 1 to 7 days and 18 h light and 6 h dark from 8 days to slaughtering age. Standard group (STD) had a stocking density of 19 birds/sq m (19,000/poultry house), 2.5 kg/sq m of wood shaving as litter and a light regimen of 23 h light and 1 h dark from 1 day to slaughtering age. A summary of details of the conditions used in this trial are reported in table 1.

The 20% birds for each group were processed at 1.7 kg of live weight (40 d of age) avoiding the stocking density exceeding 25 kg/sq m and 32 kg/sq m for WLF and STD, respectively. The trial lasted 50 days. Productive traits were recorded either at 40 or 50 days. Ten samples of litter per each poultry house were collected - at 40 and 50

days of rearing - in representative sites of the houses, away from feeding troughs and drinkers, and used for monitoring moisture. Litter moisture was determined by oven drying two samples of 10 g of litter at 80°C for 48 h and measuring the loss of water. Qualitative traits of carcass such as uniformity, bruises, skin damages, fatness as well as carcass yields, were evaluated either at 40 or at 50 days of age. Meat quality evaluation was carried out on 15 breast fillets (*P. major* muscle) per each group and at each slaughtering age (a total of 60). Colour was determined by using the CIE L* a* b* system with a Minolta Colorimeter CR 300; pH was evaluated at 15 min and 24 h *post mortem* using a portable pH meter (Hanna Inst.®) inserting the probe directly into the muscle; moisture was calculated by measuring the loss of water of 5 g of meat oven dried at 103°C for 20 h; water holding capacity was calculated for drip loss by weighting the loss of water of the breast fillet kept suspended for 24 h in glass box at 4°C (Honikel, 1998). Texture of oven cooked breast meat was measured by using TA-Hdi® Texture Analyser with an Allo-Kramer load cell of 250 kg with a speed rate of 500 mm/min (Papinaho and Fletcher, 1996). Low resolution Nuclear Magnetic Resonance (LR-NMR) analysis were carried out on two meat rods of 30 mm lengths and 35 mm^2 per breast and analysed at 20 MHz with a Bruker Minispec MQ 20 spectrometer. Each measurement was carried out with the standard CPMG sequence (Cremonini *et al*., 2001). The data from meat quality evaluation were submitted to one-way ANOVA testing housing conditions as main factor within each slaughter age.

Results and discussion

Although the stocking density of STD was quite higher than WLF, no difference emerged for environmental conditions such as temperature, relative humidity and litter moisture between WLF and STD. Several reports indicate that high stocking density worsens litter and environmental conditions leading to an enhanced incidence of foot-pad dermatitis (Blokhuis and Van Der Haar, 1990; Berg, 1998). In our experiment the efficient conditioning system, set to maintain the same temperature in the two poultry houses taking into account the size of flocks, cancelled the differences of heat and humidity production of birds kept at a higher stocking density.
WLF broiler grew up faster than STD reaching the scheduled live weight of 1.7 kg (small size broiler) at 40 d of age exceeding of 90 g STD birds. Because of their higher growth rate, in the second slaughtering session, scheduled for 2.3 kg of live weight (medium size broiler), they were killed one day in advance, i.e. at 49 d instead of 50 d. Nevertheless WLF weighed 124 g more than STD. WLF birds consumed 10% more feed than STD with a higher feed conversion rate. The overall mortality was identical in the two groups (Table 2).

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Carcass yields, uniformity, skin damages and fatness were not affected by housing conditions (Table 3). The percentage of birds with bruises was slightly lower in WLF group compared to STD (8% *vs* 10%).
For breast meat of birds slaughtered at 40 d, WLF had higher moisture content and lower water holding capacity tested by both drip and cooking loss methods, whereas the other meat quality traits were insensitive to the flock management (Table 4).
For breast meat of broilers processed at 49-50 d (Table 5), WLF confirmed to have a higher moisture content and drip and cooking losses. These results are consistent with the NMR parameter alpha and $T2_a$ which were significantly different between experimental groups. The water of type "a" has been associated to the protons of water less tightly bound to meat structure than water having relaxation time T_{2b}. In addition, alpha NMR provides the amount of the fraction of water with $T2_a$. These indicate that the proportion of water of type "a" is greater (higher alpha values) and much easier to be loosen during storage (higher $T2_a$ values) in WLF breast meat. These results are in accordance with Castellini *et al*. (2002) who observed in broilers kept at low stocking density with free access to external areas, lower water holding capacity ascribed to a higher motor activity. The faster *post mortem* pH fall observed in WLF might be responsible of the lighter breast meat colour as shown by their higher L* values.
In conclusion, broilers reared in improved housing conditions (lower stocking density, deeper litter) and with a light regimen close to the natural one, grow up faster, and supply meats containing more water that is easier to be loosen during storage in comparison with conventional reared broilers. Anyway, considering that animal productivity is an indicator of animal welfare conditions (Broom, 1991), we can assume that WLF birds were really kept in improved welfare conditions.

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Table 1. Characteristics of the rearing environment

	WLF	STD
Feeding trough, cm/bird (from 0 to 40 d)	1.54	1.17
Feeding trough, cm/bird (from 40 to 50 d)	1.96	1.53
Nipples, birds/nipple (from 0 to 40 d)	9.7	13
Nipples, birds/nipple (from 40 to 50 d)	7.5	9.9
Litter (wood shavings), kg/sq m	3.5	2.5
Light, h light/d (from 0 to 7 d)	23	23
Light, h light/d (from 8 to 50 d)	18	23
Housed chicks, n./house	14,000	19,000
Bird density at 0 d, n./sq m	14	19

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Table 2. Productive traits of light and medium size broiler chickens

	WLF	STD
Live weight at 40 d (g)	1,768	1,677
Live weight at 49-50 d (g)	2,331	2,207
Stocking density at 40 d (kg/sq m)	24.35	31.18
Stocking density at 49-50 d (kg/sq m)	24.89	31.58
Feed consumption from 0 to 49-50 d (g/bird/d)	92.85	83.99
FCR from 0 to 49-50 d (kg/kg)	1.95	1.90
Mortality from 0 to 40 d (%)	1.61	2.15
Mortality from 0 to 49-50 d (%)	2.41	2.53

Table 3. Slaughtering results of broiler chickens

	WLF (49 d)	STD (50 d)
Live weight (g)	2,331	2,207
Carcass weight (g)	1,627	1,568
Slaughtering yields (%)	69.80	71.03
Breast (%)	26.85	26.89
Thigh + drumstick (%)	45.54	44.83
Wings (%)	9.04	8.51

Table 4. Meat quality traits of light broiler chickens (40 d)

N = 15	WLF	STD	P
pH 15 min	6.09	6.06	0.62
pH 24 h	5.90	5.87	0.44
L*	54.28	53.74	0.58
a*	1.85	2.20	0.30
b*	3.82	3.99	0.76
Drip loss (%)	2.41	1.75	0.00
Cooking loss (%)	18.71	15.58	0.00
Moisture (%)	74.22	73.64	0.02
Shear value (kg/g)	1.72	1.81	0.65
Alpha (%)	14.54	14.84	0.65
$T2_b$ (ms)	40.12	39.35	0.28
$T2_a$ (ms)	100.65	102.23	0.81

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Table 5. Meat quality traits of medium size broiler chickens (49-50 d)

N = 15	WLF	STD	P
pH 15 min	6.05	6.23	0.01
pH 24 h	5.98	6.01	0.21
L*	50.76	47.86	0.00
a*	1.83	2.04	0.27
b*	3.41	3.84	0.14
Drip loss (%)	1.43	1.26	0.02
Cooking loss (%)	14.49	11.21	0.00
Moisture (%)	75.07	73.77	0.00
Shear value (kg/g)	1.70	1.70	0.99
Alpha (%)	15.51	13.67	0.03
$T2_b$ (ms)	42.04	41.42	0.16
$T2_a$ (ms)	124.89	103.89	0.00

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CARCASS CHARACTERISTICS OF MALE BROILERS FROM TWO COMMERCIAL STOCKS FED EITHER RESTRICTED OR *AD LIBITUM* AND REARED AT HIGH ALTITUDE *VS* SEA LEVEL

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Keywords : Ascites, stock, altitude, carcass, yields

Abstract

Slaughter weight and relative weight of carcass, breasts, legs and abdominal fat pad to body weight in male broilers from two commercial stocks (S1 and S2) commonly used in Turkey were determined at high altitude (Van, 1720 m) and sea level (Izmir), either under with a quantitative feed restriction (FR) between 7-14 d (R7-14d) or 7-21 d (R7-21d) or *ad libitum* (AL) conditions. There were differences between stocks for slaughter weight and carcass part yields in the response to high altitude and growth restriction. Under high altitude condition, significantly higher mortality from ascites (14.49 %) was observed than that of sea level (1.62 %) and high RV/TV ratios showed increased heart load in broilers even they did not develop ascites.

Résumé

Des poulets standards mâles, issus de deux souches commerciales (S1 et S2) utilisées couramment en Turquie, ont été élevés à haute altitude (Van, 1720 m) et au niveau de la mer (İzmir), soit avec un rationnement alimentaire quantitatif (RA) entre 7 et 14 jours ou 7 et 21 jours, soit avec une alimentation *ad libitum*. A l'abattage, le poids vif, le rendement en carcasse, filets, cuisses-pilons et le pourcentage de gras abdominal par rapport au poids vif ont été déterminés. L'élevage à haute altitude et le rationnement alimentaire ont eu un effet significatif sur le poids vif à l'abattage et les rendements en découpe. A haute altitude, une mortalité considérable (14.49 %) due à l' ascite a été

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constatée par rapport à celle au niveau de la mer (1.62 %). Les ratios RV/TV élevés ont montré que le rythme cardiaque augmentait même chez les poulets qui n'étaient pas atteints d' ascite.

Introduction

Growth restriction by food restriction is considered as a management tool for reducing mortality due to ascites and sudden death syndrome in broilers (Julian, 1993; Gonzales et al., 1998, Lopez Colello et al. 2000). However, the use of this management technique depends on the body weight at a certain slaughter age and improvements in carcass yields. There have been conflicted results in catch-up growth, proportional breast weight and abdominal fat weights due to severity and duration of the growth restriction. Some workers pointed out that feed restriction resulted in lower carcass and abdominal fat pad (Plavnik and Hurwitz, 1985; Jones and Farrel 1992) while a number of studies showed no effect on fat deposition (Altan et al., 1998; Lee and Leeson, 2001). It reduces slaughter weight and leads to lower yields especially in breast muscle (Acar et al. 1995). High altitude increases ascites incidence and susceptibility to ascites differs between strains (Huchzermeyer et al., 1988). Thus, the objective of this study was to determine the yield of carcass and some carcass parts of male broilers from two commercial stocks subjected to either restricted or *ad libitum* feeding and reared at high altitude *vs* sea level.

Material and Methods

Stocks were obtained from a commercial hatchery plant at sea level. Chicks were transported to high altitude by airway in the same day. During the rearing period, standard housing and management procedure applied at each altitude. Stock density was 13 birds per square meter for each altitude**.** Commercial broiler rations were supplied to each altitude from the same commercial feed integration. Dead birds were subjected to *postmortem* examination from 3 wk to the end of the experiment to determine the cause of death.

At 48 d of age, 12 males from each commercial stock and feeding group were randomly chosen from each altitude. 10 h of feed deprivation were applied to the birds and all the birds were weighed before processing at University's experimental laboratories at both locations. After bleeding, scalding, plucking and evisceration, hot carcasses were weighed and cut into parts. Weights of breast (skin and bones included) and legs (thighs and drumsticks, skin and bones included), and abdominal fat pad (AF)

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were recorded and presented as a ratio of body weight. Hearts were dissected and RV/TV (in full) ratios were measured. Data were analysed using GLM procedure of JMP statistic package (SAS Inst., 2000) with stock, altitude, feeding main effects and all possible interactions. Means were separated using t-test.

Results and Discussion

Significantly higher ($P<0.05$) ascites mortality was observed at high altitude (14.46%) compare to the sea level (1.62%) but rate of mortality from ascites did not differ with stock. Distribution of mortality in the stock and feeding groups at sea level and at high altitude are presented in Table 1. Significantly higher RV/TV ratios at high altitude (Table 2) accompanied by high ascites mortality were observed. This result clearly showed increased load on the heart of the broilers due to low O_2 partial pressure, even if they have not developed ascites (Julian, 1993; Balog et al., 2000; Wideman, 2001). Feeding significantly affected total mortality rate at both altitude ($P<0.03$) but mortality due to ascites differed with feeding at high altitude only ($P<0.05$). The highest ascites mortality was seen in R7-14d group. This was probably related to increased O_2 demands of restricted broiler during re-alimentation, and low O_2 pressure at high altitude. At high altitude BW significantly depressed as expected (Balog et al., 2000). However, responses of the stocks under growth restriction significantly differed with altitude that results in significant stock X feeding, stock X altitude and stock X altitude X feeding interaction effects (Table 3). S1, R7-14d group compensated growth retardation at the sea level, however, both restricted groups from S1 had lower weights than AL at high altitude. None of the restricted groups from S2 showed catch-up growth at sea level, but, R7-14 group was heavier than that of AL and R7-21 groups at high altitude suggesting that, at high altitude, one week of feed restriction may be used for S2 stock.

High altitude reduced breast part weight as percentage of BW ($P<0.05$). Stock by feeding interaction effect was significant on relative breast weight ($P<0.05$), and there was no significant difference between AL and restricted groups in S2 for relative breast weight. However, both restricted groups from S1 had significantly lower breast proportions than that of AL fed ones with in convenience to the reports of Acar et al. (1995) and Balog et al. (2000). High altitude also reduced the carcass yield and AF ratio to BW. Significant altitude, by stock interaction, effect was observed for carcass and AF percentages. S1 had lower relative AF weight and carcass yield than S2 at sea level, but, at high altitude, ratios of stocks were similar (Table 4). For carcass yield, significant stock by feeding interaction resulted in no difference between AL and restricted groups in S2, while both restricted groups from S1 had significantly lower carcass yields than that of AL fed ones (Table 5).

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Feeding did not affect AF ratios in the experiment and none of the effects interact with feeding regimen on the contrary to Plavnik and Hurwitz (1985) and Jones and Farrel (1992). However, no effect of early feed restriction on AF ratio at 49 d of ages (Altan et al., 1998) and even higher abdominal fat ratios in restricted broilers (Acar et al. 1995) have been reported in previous works.
From the present results, it may be speculated that growth depression due to high altitude was more pronounced in S2 with the exception of R7-14d FR group. As a result, growth acceleration of the stocks has to be considered before feed restriction manipulation applied to broilers to reduce the mortality due to ascites. Reductions in the breast part due to feed restriction may also differ with stock.

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Table 1. Mortality rates of stocks and feeding groups at sea level and high altitude

	Sea Level		High Altitude	
Effect	Total Mortality (%)	Ascites Mortality (%)	Total Mortality (%)	Ascites Mortality (%)
Stock				
S1	1.50 (2/133)	1.50 (2/133)	17.36 (25/144)	15.28 (22/144)
S2	10.17 (24/236)	1.69 (4/236)	16.34 (42/257)	14.01 (36/257)
Probability	<0.01	N.S.	N.S.	N.S.
Feeding				
AL	11.86 (4/118)	2.54 (3/118)	12.16 (18/148)	10.81 (16/148)
R7-14d	3.10 (4/129)	1.55 (2/129)	24.00 (30/125)	20.80 (26/125)
R7-21d	6.56 (8/122)	0.82 (1/122)	14.84 (19/128)	12.50 (16/128)
Probability	0.03	N.S.	0.03	0.05

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Table 2. Effects of Stock, Altitude and Feeding on Live Body Weight, Carcass Yield and Relative Weight of Carcass Parts to Body Weight, %

	BW	% Carcass	% Breast	% Legs	% Ab. Pad	RV/TV
Stock						
S1	2711±18	73.93±0.24	22.04±0.19	21.48±0.17	1.05±0.04	0.28±0.01
S2	2631±19	74.28±0.25	23.11±0.20	20.99±0.18	1.16±0.04	0.27±0.01
Altitude						
Sea Level	2795±19	75.75±0.25	22.87±0.20	22.00±0.18	1.19±0.04	0.21±0.01
High	2547±19	72.46±0.25	22.28±0.20	20.40±0.17	1.02±0.04	0.34±0.01
Feeding						
AL	2800±23 [a]	74.90±0.30[a]	22.87±0.25 [a]	21.66±0.21	1.12±0.05	0.27±0.01
R7-14d	2766±23 [a]	73.94±0.30[b]	22.80±0.24 [a]	21.00±0.21	1.18±0.05	0.28±0.01
R7-21d	2447±23 [b]	73.48±0.30[b]	22.05±0.24 [b]	21.08±0.21	1.03±0.05	0.27±0.01
Variation Sources	**Probability**					
Stock	0.003	0.307	<0.001	0.055	0.062	0320
Altitude	<0.001	<0.001	0.037	<0.001	0.004	<0.001
Feeding	<0.001	0.004	0.031	0.097	0.109	0.868
AltitudexStock	0.004	0.048	0.859	0.224	0.026	0721
AltitudexFeeding	0.022	0.434	0.111	0.412	0.192	0989
Stockx Feeding	0.012	0.024	0.003	0.687	0.949	0311
StockxAlt.xFeeding	<0.001	0.593	0.244	0.556	0.695	0553

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Table 3. Means for interaction effects on BW (g)

BW					
Feeding		AL	R7-14	R7-21	**StockXAltitude interaction**
S1	Sea Level	2905±46^{ab}	2918±44^{ab}	2565±43^{d}	**2796±29^{x}**
	High Altitude	289±144^{b}	2641±46^{cd}	2348±44^{e}	**2626±26^{y}**
StockXFeeding İnteraction		**2898±32^{A}**	**2779±32^{B}**	**2457±31^{C}**	
S2	Sea Level	3034±51^{a}	2767±46^{c}	2582±46^{d}	**2794±28^{x}**
	High Altitude	2372±44^{e}	2739±49^{c}	2293±49^{e}	**2468±27^{z}**
StockXFeeding İnteraction		**2703±34^{B}**	**2753±34^{B}**	**2437±34^{C}**	

a,b,c,d: Lsmeans bearing different superscripts differ significantly; x,y,z: different superscripts show the difference between means of stock by altitude interaction; A,B, C: different superscripts show the difference between means of stock by feeding interaction (P<0.05).

Table 4. The Effect of Altitude x Stock interaction on BW (g) and abdominal fat weight (%)

Altitude / Stock	N	Carcass	Abdominal Fat
Sea Level			
S1	36	75.22±0.33^{b}	1.07±0.06^{a}
S2	32	76.26±0.36^{a}	1.32±0.06^{b}
High Altitude			
S1	35	72.63±0.34^{b}	1.03±0.06^{a}
S2	32	72.30±0.36^{a}	1.00±0.06^{a}

a,b: Lsmeans bearing different superscripts in each column differ significantly (P<0.05)

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Table 5. The Effect of Stock x Feeding interaction on BW (g), carcass yield and relative breast weight (%)

Stock / Feeding	N	% Carcass	% Breast
S1			
AL	23	75.39±0.42 [a]	23.03±0.33[a]
R7-14d	23	73.53±0.42 [b]	21.96±0.33 [b]
R7-21d	25	72.86±0.40 [b]	21.13±0.32 [b]
S2			
AL	22	74.40±0.45 [a]	22.71±0.36 [a]
R7-14d	21	74.35±0.44 [a]	23.65±0.35 [a]
R7-21d	21	74.10±0.44 [a]	22.98±0.35 [a]

a,b: Lsmeans bearing different superscripts in each column differ significantly ($P<0.05$)

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MEAT QUALITY OF BROILERS FROM THE ORGANIC PRODUCTION

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Keywords : Carcass composition, meat quality, organic production, broiler

Abstract

In a study from 5 test series (n = 7.864) broiler were held under ecological conditions and compared with a control group. Thereby the fattening duration varied from 35 (control group) to 80 days. Following broiler genotypes were used: Ross 308 (control group), ISA J 457/ISA J 257, ISA JA 57, RedBro (Shaver) as well as Sena (double Breast) and Ross (Mini). For the registration of the carcass and meat quality 380 broiler were investigated in the laboratory. The investigations led to following results: Large differences are to be found in the composition of the carcass, particularly the tissue proportions of the joints breast and thighs; the chemical composition of the breast- and thigh meat was subject to the influence of the production process; there was no significant improvement in the sensory characteristics of breast - and thigh meat through the ecological production in comparison to the conventional one; the influence of the fattening age and the breed was statistically demonstrable.

Résumé

Dans une étude réalisée sur 5 lots (n=7864), des poulets de chair ont été élevés suivant le concept de l'agriculture biologique ou suivant des méthodes standards. Les génotypes suivants on été utilisés : Ross 308 (groupe standard), ISA J457/ISA J257, ISA JA57, RedBro (Shaver) ainsi que Sena (large filet) et Ross (Mini). Pour les mesures qui concernent la carcasse et la qualité de la viande, 380 animaux ont été étudiés au laboratoire. Ces mesures donnent les résultats suivants : De larges différences existent dans la composition de la carcasse, en particulier au niveau du pourcentage de l'ensemble filet + cuisses. La composition chimique de la viande du filet et des cuisses est influencée par le mode de production. Il n'y pas d'amélioration des caractéristiques sensorielles de la viande du filet ou des cuisses avec le mode de

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production biologique. Par contre l'influence de l'âge et de la souche est statistiquement significative.

Introduction

The EU regulation on organic production [Regulation (EC) No. 1804/1999] prescribes the conditions of the production. Furthermore, following demands on the organic production are put in the guidelines of ecological associations in Germany (Bioland, Naturland): slowly growing breeds are to be used, grain share in the ration should be at least 65 %, also the rearing duration, trimming density and open yard size are set.

Material and methods

At an animal material of broilers from 5 test series (n = 7864) the animals were held under controlled ecological conditions and compared with a (conventional) control group. The rearing duration varied from 35 (control group) to 80 days. Thereby following breeds were included: Ross 308 as control group, rearing duration 35 days (n = 4000); Sena, rearing duration 60 days (n = 864); ISA J 457 brown feathered broiler as well as ISA J 257 white feathered broiler with 77 days (n = 2000) and also slowly growing hybrids ISA JA 57 and RedBro (Shaver) and quickly growing breeds Sena and Ross with a rearing duration of 80 days (n = 1000). The comprehensive data about feeding and keeping can be taken from RISTIC and DAMME (1996, 1999, 2002). The growing performance of the animals was determined too. A total of 380 carcasses were analyzed on their carcass composition and meat quality. The used methods are described by RISTIC et al. (1994) and RISTIC and FREUDENREICH (2000). The statistical evaluation occurred with a SAS - and SPSS (ANOVA)-program package using a fixed model. The multiple median value comparison was accomplished with the help of the Tukey-test ($p \leq 0.05$).

Results and discussion

The development of the weight was determined first of all by the duration of the rearing period (Tab. 1). In each growing period an other breed was represented. A clear delimitation resulted at equal fattening age after 70 days between slowly- and fast growing broiler lines (ISA, RedBro to Sena, Ross). Thereby a difference of 1325 g

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was found in favour of the breeds Sena and Ross and the feed utilisation was more favourable by 120 g.

Tab. 1: growing performances of the different breeds (n = 7864)

Rearing duration/days	35	56	77	70[1)]	70[2)]
Weight g	1675	2455	2708	2250	3575
Feed utilisation (g feed/kg increase)	1717	2243	2489	2735	2615
Losses %	4.7	4.6	5.0	4.3	6.1

1) slowly growing breeds (ISA, RedBro)
2) fast growing breeds (Sena, Ross)

The slaughter weight of chickens from the traditional production was approximately of. 1200 g, while that from the ecological production ranged from 1800 to approx. 3000 g (Tab. 2). The share of the valuable joints was between 27 and 31 % (breast) and between 30 and 32 % (thigh). The fattiness of the carcass by means of the abdominal fat ranged between 2.3 and 4 %. The lowest significant difference was 0.8 %.
The supreme meat quantity of the both valuable joints had the Sena and Ross broiler, as well as the broiler from the conventional production (Tab. 3). The highest total amount of fat, calculated from the tissue dissection of the parts breast and thighs summed up with abdominal fat was found to be 7.4%, after 77-day or rearing period for ISA–broilers.
The lipid content of the breast meat of broilers from the conventional production was 0.9 % (Tab. 4). The lipid value of broilers from ecological production were for the most cases lower with one exception of the ISA - broiler after 77-day of rearing (1.03 %). Despite that the water, protein and ash content values of the breast meat showed statistically significant differences, it was noticeable that these values were in a narrow range.
For the sensory evaluation of the breast meat, the duration of the rearing was always significant (Tab. 5). Larger differences appeared for juiciness and tenderness between the different growing duration and thus between the examined breeds. The best scores in juiciness were for the Ross - broiler after a 35-day rearing period, followed by Sena - broilers after a growing duration of 60 days, which achieved at the same time also higher notes for tenderness with 5.4. For flavour and overall impression of the breast meat there was a similar tendency as for the scores of juiciness and tenderness.

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After a short fattening period of 32, 35 and 39 days average final weights of 1590 g, 1775 g and 2206 g were achieved respectively, at breeds from the German market (DAMME and RYCHLIK, 2001; SIMON. 2001): . The feed conversion index were 1:1.519, 1.618 and 1.705 respectively.

A clear difference existed for the live weight of the different breeding products, like e.g. Ross 308 to ISA S 657 (GRASHORN and CLOSTERMANN, 2002). By using regression analysis it was demonstrated , that the slowly growing breeds must be reared 10 to 32 days longer, to reach a live weight of 2000 g, as the breed Ross 308 on the 42nd day. In the present investigation this development was demonstrated.

The abdominal fat content was for the broilers from the traditional production as well as for slowly growing breeds (ISA, RedBro) lower than for the other breeds. After a rearing period of 70 and 84 days, a similar tendency was found by GRASHORN and CLOSTERMANN (2002).

The proportion of the valuable joints breast and thighs grows in principle with the age (HAVENSTEIN et al., 1994; LEWIS, et al., 1997; RISTIC, 1991, 1992, 1994, 2002For slow growing breeds it is not always the case (GRASSHORN and CLOSTERMANN, 2002). Similar observation was made in the present investigation, because ISA J 457/J 257 as well as ISA JA 57 had a lower proportion of the joints breast and thighs. The slowly growing breeds had a higher thigh percentage than that of the fast growing breeds (LEWIS at al., 1997).. Slow growing breeds have a lower breast meat percentage than fast growing breeds (LEWIS et al., 1997; HAVENSTEIN et al., 1997).

The nutrient content of the meat, particularly in the thigh muscles changed also with age. The water and protein contents decreased while the fat content increased. The fat content of the breast meat from the traditional production is approx. 0.5 %. The corresponding value from the biological production is of the same level. With the organic feeding, the fat content of the thigh meat has grown dramatically but with no effects on the sensory characteristics (RISTIC, 2000). According to CULIOLI et al. (1990) it is supposed, that a higher fat content in the muscle tissue lead to better flavour characteristics of the meat in slow growing broiler. With increasing rearing duration, the sensory properties (juiciness, tenderness) were improved in from 5 to 8 weeks in commercial production conditions (RISTIC, 1994). An improvement appeared also at the breed “Landkornhähnchen”"" with 46 days, which had an intramuscular fat content of 0.17 % in the breast meat. The juiciness and tenderness scores of the breast meat, in comparison with chickens from the traditional production, were significantly higher of about 0.7 points (4.0:4.7) and 0.4 points (4.9:5.3) respectively (n = 110). The sensory scores of the commercial group in the present investigation were superior when compared with the data from the biological production of the slow growing lines. The fast growing lines obtained better results.

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Tab. 2: Carcass composition (n = 380)

Rearing duration (days)	Body weight, g	breast % BW	Thighs % BW	Abdominal-fat, % BW
35	1198	29,3	30,4	2,3
60	2019	29,2	31,0	2,6
77	1929	27,6	29,5	4,0
80 [1)]	1816	27,1	31,7	2,6
80 [2)]	2985	30,7	30,5	3,4
significance	***	***	***	***
$LD_{0,05}$ [3)]	113	1,4	0,9	0,8

[1)] slow growing lines (ISA, RedBro)
[2)] fast growing lines (Sena, Ross)
[3)] $LD_{0.05}$ = lowest significant difference at $p \leq 0.05$

Tab. 3: Tissue proportions of the joints breast and thighs

Rearing duration (days)	meat g	fat g	meat % BW	fat % B	Σ fat % BW
35	494	35	41,2	2,9	5,2
60	828	57	41,0	2,8	5,4
77	747	65	38,7	3,3	7,4
80	705	39	38,9	2,1	4,7
80	1261	95	42,1	3,2	6,6
significance	***	***	***	***	***
$LD_{0,05}$	81	18	0,9	0,7	1,9

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Tab. 4: Chemical composition of the breast meat (% of fresh weight)

Rearing duration (days)	fat	water	protein	ash
35	0,86	74,3	24,0	1,1
60	0,36	74,9	23,9	1,2
77	1,03	74,4	23,6	1,0
80	0,46	74,9	23,9	1,3
80	0,44	74,7	24,1	1,2
significance	***	***	***	***
$LD_{0,05}$	0,4	0,1	0,3	0,1

Tab. 5: Sensory data of the breast meat[1)]

Fattening duration (days)	juciness	tenderness	aroma	total-impression
35	4,5	5,2	4,7	4,7
60	4,4	5,4	4,4	4,5
80	3,8	5,0	4,2	4,3
80	4,2	5,3	4,4	4,5
significance	**	***	*	*
$LD_{0,05}$	0,4	0,3	0,5	0,2

[1)] semantic Scale ranges from 1 (very unsatisfactory) to 6 (excellent)

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THE EFFECTS OF CHANGES IN ENVIRONMENTAL CONDITIONS WITHIN POULTRY TRANSPORT VEHICLE ON BLOOD PARAMETERS

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Keywords : broilers, transportation stress, vibration, blood parameters

Abstract

The variability in broiler transportation systems between countries is well known. The objective of this study was to investigate the changes in temperature, relative humidity and vibration parameters within poultry transport vehicle during a 1.5 h journey in Turkey. Physiological blood parameters of broilers were also determined.
Temperature and humidity ranged between 20.6 and 24.2ºC and 61 and 69%, respectively. The acceleration value ranged from 0.04 to 0.52 m/s^2 and clustered around 0.25 m/s^2. The highest acceleration levels were obtained when vibrations frequency ranged between 3 and 30 Hz. Plasma creatine kinase activity, uric acid, albumin and lactic dehydrogenase activity increased after journey.

Résumé

Il est bien établi que lessystèmes de transport des poulets varient d'un pays à l'autre. L'objectif de cette étude était d'examiner les changements de température, d'humidité relative et de vibration dans les camions pendant une heure et demi de voyage. Des paramètres physiologiques sanguins ont été également déterminés. La température et l'humidité variaient entre 20.6 et 2.2°C et entre 61 et 69%, respectivement. L'accélération variait entre 0.04 et 0.52 m/s² la valeur moyenne se situait autour de 0.25 m/s². Le niveau maximum d'accélération était concomitant à des fréquences comprises entre 3 et 30 Hz. Dans le plasma, l'activité de la creatine kinase, l'acide urique, l'albumine et l'activité de la lactate dehydrogenase ont augmenté au cours du transport.

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Introduction

Prior to slaughter, broilers may be exposed to a number of stressors. The process of handling, crating and transportation can cause injuries, which reduce the birds' well-being and increase economic losses (Kanan and Mench, 1996; Nicol and Scott, 1990). Among these stressors, one of the major sources of potential stress, is the transportation microenvironment (Kettlewell *et al.,* 1993). Temperature and humidity in vehicle, noise and vibration of the vehicle seem to be stressful aspects of transportation and result in physiological and biological changes in birds (Weeks *et al*., 1997; Mitchell and Kettlewell, 1998). Mitchell *et al* (1992) found changes in heterophil:lymphocyte ratios and an increase in plasma creatine kinase in broilers after transportation. Freeman *et al.* (1984) reported that a 2-4 h transport induced hyperlipidemia and hyperglycaemia in broilers.

The vibration frequency in transport vehicles may also induce changes in blood flow, blood circulation and heart beat (Scott, 1994). Randall *et al* (1994) reported that frequency in the vertical axis of 1-3 Hz was evident and, in addition, a resonant frequency of 10Hz occurred.

Biochemical changes resulting from transportation could affect product quality. High vibration during transportation resulted in reduced glycogen concentration in liver and in muscle *biceps femoris* (Warris *et al*., 1997). Transport stress-induced tissue dysfunction and damage are reflected by increased plasma activity of intracellular muscle enzymes, including creatine kinase (Mitchell *et al*., 1992).

In Turkey, 611-tone broiler meat is produced and annual consumption is about 10 kg per capita. The variability of broiler transportation systems between countries is well known. The objective of this study was to investigate the changes in temperature, relative humidity, noise and vibration parameters within poultry transport vehicle during a 1.5 h journey in Turkey. Physiological blood parameters of broilers were also determined.

Material and Methods

This study was performed under commercial conditions. Broilers were reared until 42 d of age. A commercial starter diet with 235 g CP/kg and 13.05 MJ ME/kg from 0 to 14 days of age, a grower diet with 225 g CP/kg and 13.10 MJ ME/kg from 14 to 28 days and a finisher diet with 205 g CP/kg and 13.56 MJ ME/kg from 29 to 42 days were provided for *ad libitum* consumption. The light regimen was 23 h light : 1 h darkness throughout the breeding period.

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At 42 d of age food was withdrawn for 3 h and all broilers were manually caught and placed in plastic crates (80x60x33cm). The number of birds per crate was 10. A total of 252 crates (2520 broilers) were loaded onto the vehicle. The average broiler weight was 1980g and approximately 5 tons broilers were transported.

Physiological measurements

Ten broilers were randomly selected and the initial blood samples were obtained as soon as the broilers were crated. Blood samples were collected from the left branchial vein into tubes containing EDTA. Plasma samples were obtained by centrifugation of blood at 1500Xg for 5 minutes and stored at –20ºC until analysis of creatine kinase, albumin and uric aside and Lactade dehydrogenase (LDH). Blood samples were taken from ten broilers randomly selected after 1.5 h of journey.

Measurements during transportation

The truck which has suspension system with spring leaf and pneumatic shock absorber (air suspension system) was used for transportation (Figure 1). Truck speed was about 70 km per hour.

Figure 1. View of the truck suspension system

Noise and Vibration Measurement System

Portable vibration and noise measurement system which consists of an accelerometer, a microphone, a data logger, a power supply, a communication module, a PULSE Software (Brüel & Kjaer-Denmark), a connection cable and a magnet was used to obtain vibration (acceleration and frequency) and sound data related to road and vehicle conditions during transportation (Figure 2).

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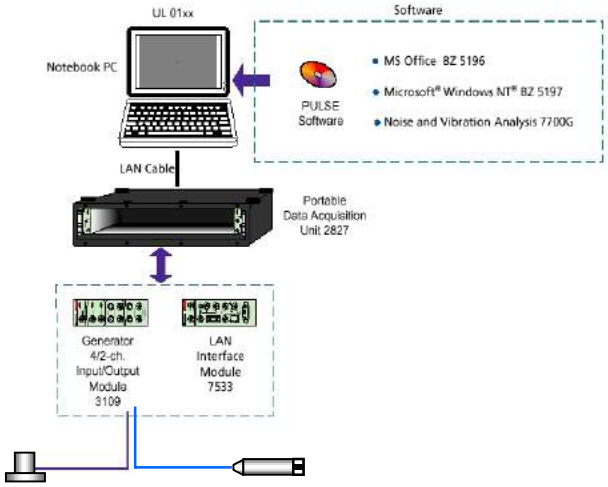

Figure 2. Noise and Vibration measuring system

The measurements were carried out every 5 minutes in order to have a sensitive information concerning road conditions.
A digital thermometer and an Elimko 6000 Higrometer which was located among cages on the truck were used to measure temperature and relative humidity. Temperature and humidity were recorded every two minutes during the transportation.
Statistical analysis: The data on blood parameters were analyzed using GLM procedure of SAS (SAS Inst., 1989).

Results and Discussion

Each measurement of vibration value was shown with different color in Figure 3. The acceleration value ranged between 0.04 and 0.52 m/s^2 and clustered around 0.25 m/s^2. Alayunt *et al.* (2003) reported that the highest displacement during the transportation was obtained at the lower frequency values. In the present experiment, the highest acceleration levels were obtained when frequency ranged between 3 and 30 Hz (Figure 3).
Temperature and humidity ranged between 20.6 and 24.2ºC. Thermal zone of broilers weighing 1.6 kg is between 23 and 28ºC (Meltzer, 1983). Etches *et al.* (1995) reported that broilers heat stressed when temperatures reach 28ºC. Although broilers in this

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experiment were heavier than 1.6kg, the temperatures in vehicle were not enough to induce heat stress. The relative humidity was between 61 and 69 % (Figure 4).

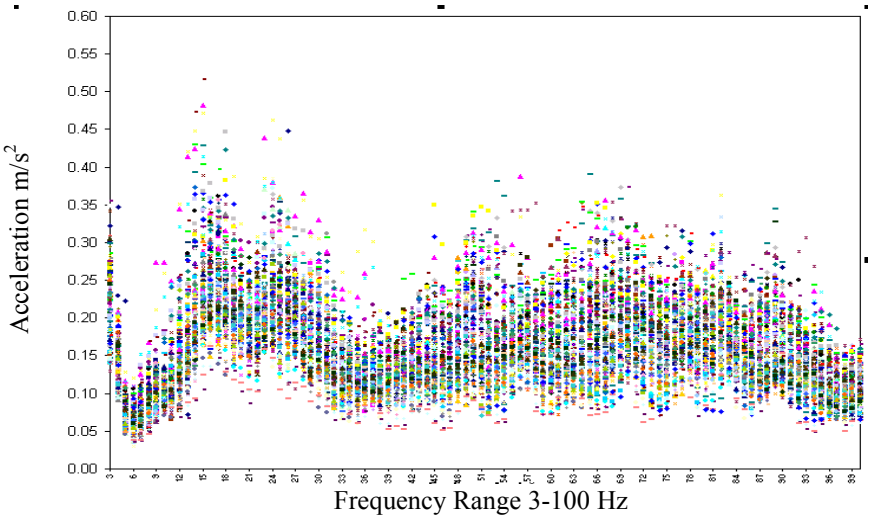

Figure 3.

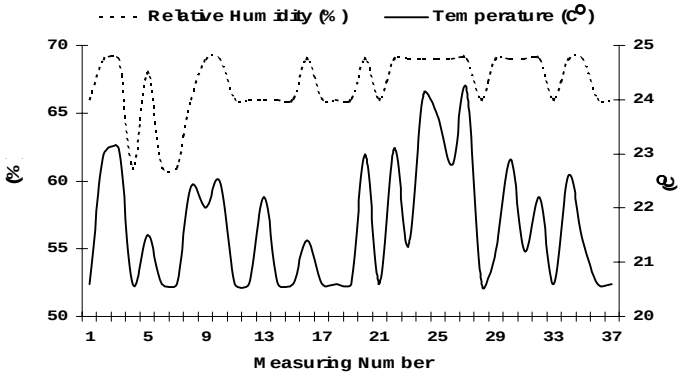

Figure 4. Relative humidities and temperatures during transportation.

Due to the road conditions, the level of noise ranged between 77 dB (A) and 92 dB (A).

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Table 1. Blood parameters before and after transportation.

	LDH U/L	Uric acid mg/dl	Creatine kinase U/L	Albumin g/100ml
Before transportation	2166	4.20	4130	1.53
After transportation	3172	7.20	10420	1.74
Standard error	120	0.51	160	0.06
Significance (P value)	0.043	<0.001	<0.001	0.031

The levels of LDH, uric acid, creatine kinase and albumin increased significantly after a 1.5 h journey (Table 1). The increment in creatine kinase level was in agreement with Mitchell *et al.* (1992). The high creatine kinase level indicates significant muscle damage. Energy metabolism was also affected, as indicated by the increase in uric acid level (Siegel and Van Kampen, 1984).

In conclusion, the temperature and humidity to which birds were exposed during transportation did not result in a thermal load in spring in Turkey. Thus, we concluded that changes in blood LDH, uric acid, creatine kinase activity and albumin levels could be due to transport conditions rather than thermal load. This result is in agreement with previous observations of Abeyesinghe *et al* (2001). Scott (1994) reported that the fundamental frequency of poultry transporters was 1 and 2 Hz. The highest displacement occurs between 3 and 7 Hz frequency (Marcondes *et al*., 1989), which results in a decrease in vegetables and fruits product quality (Marcondes *et al*., 1989) but also damaged human health (Bjerninger, 1966). In the present study, highest displacement was obtained at 3 Hz level of frequency. Thus, suspension systems of vehicle should be improved to prevent adverse effect of transportation on broilers.

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EFFECT OF SLAUGHTERING AGE ON CARCASS CHARACTERISTICS OF BROILERS UNDER TROPICAL CONDITIONS OF SRI LANKA

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Keywords: Poultry meat, Broilers, Carcass quality, Sri Lanka

Abstract

A study was carried out to determine the effect of slaughter age on live weight, percentages of body portions of broilers, and net profit under tropical conditions of Sri Lanka. A commercial broiler strain was raised under intensive management with standard formulated feed in Dry Zone (temperature: 25-40°C; RH: 70-80%) of Sri Lanka. At the age of slaughter (ranging from 34 to 45 days), 360 birds were processed and body parts were measured separately. Linear regression analysis revealed that with the increase in slaughtering age, live weight, percentages of featherless and dressing, increased while percentages of head, neck, toes and giblets decreased significantly. With age, breast, wing and leg (thigh and drumsticks) portions showed no significant difference. Optimum slaughter age that maximized net profit was 42 days.

Résumé

L'objectif de cette étude était de déterminer l'effet de l'âge à l'abattage sur le poids vif, les rendements en découpe et le résultat économique en élevage de poulets dans des conditions tropicales du Sri Lanka. Une souche commerciale de poulet de chair a été élevée de façon intensive avec un aliment standard en zone sèche (température : 25-40°C ; HR : 70-80 %) du Sri Lanka. A l'âge d'abattage (variant de 34 à 45 jours), 360 poulets ont été abattus et découpés. Une analyse de régression linéaire a montré que l'accroissement de l'âge à l'abattage se traduisait par une augmentation du poids vif et du rendement en carcasse et une diminution des pourcentages de tête, cou et pattes. Les rendements en filets, ailes et cuisses-pilons ne sont pas affectés. L'âge optimal à l'abattage permettant d'optimiser le résultat économique est de 42 jours.

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Introduction

Slaughtering age is one of the most important factors affecting the market weight of broilers, weight of body portions, feed and other costs and ultimately profit of the broiler enterprise. Poultry breeders in Sri Lanka import broiler parent stocks directly from the major breeding companies in Canada, USA, U.K., Holland, and Germany, which maintain high quality grand-parent stocks. Sri Lankan breeder companies make the necessary two-way cross from the parent stocks to produce high quality fast growing broilers. A major problem that Sri Lankan broiler industry has to cope with is the inherent hot and humid climate prevailing throughout the year. Particularly in the Dry Zone of Sri Lanka temperature can often go up to 38-40°C during the day time with relative humidity of 70-80% leading to heat stress even under intensive management conditions. Thus, it is vital to investigate how the improved strains of temperate countries perform under the stressful tropical weather. Age of slaughter of broilers in Sri Lanka often varies from 34 days to 48 days. With the slaughtering age, percentage of body portions, input costs and profit may vary. Present study was conducted to determine the effect of slaughtering age on live weight and percentages of featherless, dressing, head, breast, legs (thighs and drumsticks), wings, giblets (liver, heart and gizzard), neck, and toes of broilers under tropical conditions in Sri Lanka. Attempts were also made to estimate the optimum age of slaughter under these conditions.

Material and Methods

In this experiment, 360 broiler birds 34 to 45 days of age were selected randomly from 27 farmers having the same strain. Grand-parent stocks of the birds belonged to a famous breeding company in Canada. Male: female ratio of the birds was about 1:1. The selected birds (undergone 8 hours of starvation) were transported to the processing plant (approximately 4 km distance), weighed and subjected to standard steps of processing. The weights of whole carcass, breast, thighs, wings, legs, drumsticks, gizzard, liver plus heart, gut, toes, head, and neck of individual birds were measured separately. Percentages of the traits were calculated with respect to live weight. In addition, data on feed cost, drug cost, total cost and net profit were collected from the farmers. Analysis of variance procedure followed by Duncan's New Multiple Range Test (DNMRT) was carried out for each trait separately to statistically compare the means of different age groups. Linear regression analysis was conducted between slaughter age versus each of the traits measured, to find out the significance of the rate of change in each trait during the period.

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Results and discussion

Tables 1, 2 and 3 summarize the means of each trait for different ages of slaughter. These values were in agreement with those observed in similar studies (Bajwa et al., 1999; Moran and Orr, 1969; Orr et al., 1984) except for the breast %, which was slightly higher in our study. The breast in our study (expressed as a percentage of live weight) included sternum and the ribs distributed on both sides of it, together with surrounding musculature (such as pectoralis muscles). Minor variations in processing may also have contributed to the slightly higher values. However, these results proved that the commercial strain used could produce 2 kg of live weight with a dressing % of about 70% in 43 days under the conditions given. Thus the performance of the improved strain under tropical conditions seemed satisfactory. Regression analysis showed that with the increasing slaughter age, percentages of featherless, dressing and gizzard increased significantly ($P<0.05$). However, percentages of head, neck, toes, liver plus heart, and giblets as a whole decreased significantly ($P<0.05$). None of these changes were very consistent or dramatic. Percentages of breast, thighs, drumsticks or wings showed no significant change with increasing slaughter age. But, feed and other costs increased significantly with age of slaughter ($P<0.05$). However, profit per bird also increased with age. Feed cost was about 68 % of the total cost. According this study, the optimum age of slaughter, which would maximize the net profit, was 42 days.

Table 1. Mean comparison of live weight, and percentages of featherless, dressing, head and breast portions at different slaughter ages[1].

Age (Days)	Live weight (kg)	Featherless	Dressing	Head	Breast
34	1.4766^{f}	90.21^{bc}	68.36^{bc}	3.16^{b}	27.42^{ab}
35	1.3266^{g}	90.72^{abc}	68.06^{c}	3.20^{b}	26.81^{ab}
36	1.5700^{ef}	89.15^{c}	68.25^{bc}	2.80^{c}	26.08^{b}
37	1.7900^{bc}	89.01^{c}	67.20^{c}	2.11^{gh}	26.40^{ab}
38	1.6650^{de}	88.97^{c}	68.00^{c}	2.20^{fg}	26.44^{ab}
39	1.6620^{de}	91.05^{abc}	68.52^{bc}	2.71^{cd}	26.26^{b}
40	1.7100^{dc}	91.20^{abc}	70.20^{abc}	3.72^{a}	26.32^{ab}
41	1.7800^{bc}	89.70^{bc}	70.21^{abc}	2.55^{cde}	25.83^{b}
42	1.8300^{b}	93.64^{a}	72.97^{a}	2.52^{de}	26.87^{ab}
43	2.0500^{a}	91.12^{abc}	68.23^{bc}	3.30^{b}	26.91^{ab}
44	2.0400^{a}	91.20^{abc}	69.28^{bc}	1.89^{h}	27.22^{ab}
45	2.0300^{a}	92.44^{ab}	71.39^{ab}	2.41^{ef}	27.90^{a}

[1]Means within each column not sharing a common superscript are significantly different ($P<0.05$) under DNMRT procedure.

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Table 2. Mean comparison of percentages of drumsticks, thighs, legs (drumsticks + thighs), wings and neck of broilers in different ages[1].

Age (Days)	Leg	Drumstick	Thigh	Wing	Neck
34	26.93^{bc}	9.98^{cd}	16.92^{cd}	7.73^{e}	2.97^{c}
35	28.34^{ab}	10.59^{abcd}	17.79^{abc}	9.29^{ab}	3.77^{a}
36	29.20^{a}	10.97^{ab}	18.22^{a}	8.24^{cde}	3.41^{b}
37	27.29^{bc}	10.12^{bcd}	17.17^{bcd}	8.03^{cde}	2.85^{cd}
38	27.09^{bc}	10.25^{bcd}	16.88^{cd}	8.38^{cde}	2.79^{cd}
39	27.08^{bc}	9.91^{cd}	17.10^{bcd}	7.81^{de}	2.93^{cd}
40	26.94^{bc}	10.19^{bcd}	16.47^{d}	8.51^{cde}	3.44^{b}
41	29.35^{a}	11.23^{a}	18.05^{ab}	9.81^{a}	2.92^{cd}
42	28.90^{a}	10.77^{abc}	18.12^{ab2}	8.61^{bcd}	2.01^{f}
43	26.67^{c}	9.98^{d}	16.79^{cd}	7.89^{de}	2.67^{d}
44	29.04^{a}	10.68^{abcd}	18.36^{a}	8.76^{bc}	2.29^{e}
45	28.34^{ab}	10.41^{abcd}	18.08^{ab}	8.61^{bcd}	3.00^{c}

[1]Means within each column not sharing a common superscript are significantly different (P<0.05) under DNMRT procedure.

Table 3. Mean comparison of percentages of liver + heart, gizzard, giblets as whole, gut and toes of broilers at different slaughtering ages[1].

Age (Days)	Liver + Heart	Gizzard	Giblet	Gut	Toe
34	3.72^{a}	3.60^{a}	10.30^{a}	7.04^{a}	4.97^{ab}
35	3.48^{ab}	3.05^{b}	10.32^{a}	6.58^{ab}	4.34^{cde}
36	2.76^{c}	2.24^{ef}	8.42^{cd}	5.82^{cd}	4.74^{abc}
37	2.28^{b}	2.49^{de}	8.63^{c}	5.79^{cd}	4.16^{de}
38	2.89 ^{c}	2.88^{bc}	6.58^{c}	5.62^{cd}	4.49^{cde}
39	2.28^{e}	2.20^{ef}	7.42^{e}	5.44^{de}	4.45^{cde}
40	2.76^{c}	2.33^{de}	8.54^{c}	5.71^{cd}	4.03^{e}
41	2.39^{de}	2.37^{de}	7.69^{e}	5.74^{cd}	4.43^{cde}
42	1.62^{f}	1.93^{f}	5.57^{f}	4.86^{e}	4.44^{cde}
43	2.67^{cd}	2.56^{cde}	7.91^{cde}	5.32^{de}	4.58^{abcd}
44	2.78^{c}	2.68^{cd}	7.76^{de}	6.91^{a}	2.61^{f}
45	3.22^{b}	3.13^{b}	9.36^{b}	6.14^{bc}	5.00^{a}

[1]Means within each column not sharing a common superscript are significantly different (P<0.05) under DNMRT procedure.

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WELFARE OF FORCE-FED DUCKS AT SLAUGHTER - EFFECT OF CURRENT INTENSITY DURING ELECTRICAL 'HEAD-ONLY' STUNNING ON EEG SPECTRUM IN THE BRAIN AND BLEEDING PARAMETERS

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Keywords : duck, head-only stunning, current intensity, spectral analysis, bleeding

Abstract

A research project has been undertaken with the aim of optimising the stunning procedure for force-fed waterfowls from the point of view of animal welfare and products quality. As a first step in this project, the present experiment investigated the effect of various current intensities during the electrical 'head-only' stunning of force-fed ducks on brain responses. Fourty six birds were surgically implanted with electrocorticographic (ECoG) electrodes, and the ECoG power spectrum was recorded before and after a 4s 'head-only' stun using a 50 Hz sinusoidal AC of 100 to 600 mA. The evolution of the relative power of the 2.5-30 Hz frequency band in the ECoG showed that a 600 mA head-only stunning followed by bleeding rendered all the birds unconscious until death, whereas some birds regained consciousness at 400 mA. The comparison of the initial rate of blood loss after a 300 or a 600 mA current stun revealed no significant difference between the treatments. The relative blood weights were however higher from 160 seconds after the neck cut with a 600 mA head-only stunning, compared to the 300 mA treatment.

Résumé

Un projet de recherche a été initié dans le but d'optimiser la méthode d'étourdissement des palmipèdes gavés, répondant à la double exigence du bien-être animal et de la qualité des produits. La première étape de l'étude s'est attachée à définir les effets de différentes intensités appliquées lors d'un étourdissement 'tête seulement' sur la réponse cérébrale de canards gavés. Quarante six animaux ont été implantés à l'aide

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d'électrodes électrocorticographiques (ECoG), et le pouvoir spectral de l'ECoG a été enregistré avant et après un choc électrique de 4 secondes, utilisant un courant alternatif sinusoïdal de 50 Hz, et d'intensité variant de 100 à 600 mA. L'évolution du pouvoir spectral de la bande de fréquence (2.5-30 Hz) de l'ECoG a montré qu'une électronarcose 'tête seulement' de 600 mA suivie de la saignée étourdissait efficacement tous les animaux, alors que certains retrouvaient la conscience après un choc de 400 mA. La comparaison des vitesses initiales de saignée après un choc de 300 ou 600 mA n'a pas montré de différence significative entre les deux traitements. Le poids relatif de sang récupéré après un choc de 600 mA était par contre plus élevé à partir de 160 secondes après la saignée qu'avec une intensité de 300 mA.

Introduction

The pre-slaughter stunning of force-fed waterfowls greatly affects the incidence of appearance defects on both fatty liver and meat. It is however a legal requirement that animals are rendered unconscious, *i.e.* insensible to pain, before death occurs from blood withdrawal. In France, the electrical stunning in a water-bath is the only method used for force-fed ducks' in commercial slaughter plants. For lean ducks, the minimum current recommended by the EU experts is 130 mA (AC of 50 Hz). However, such high a current induces various fatty liver downgrading such as red coloration of lobe tips and petechial haemorrhages, which result in strong economical losses. Therefore, studies on other stunning methods which would answer both requirements of animal welfare and products quality are particularly needed for force-ducks. Contrary to the water-bath stunning in which the applied current flows through the whole body (Woolley *et al.,* 1986), thus inducing products defects, the current crosses only the brain in the head-only stunning. This method would then be expected to improve products quality.

The observation of frequency changes in the electrocorticogram (ECoG) is a common method used for estimating depth of unconsciousness in various species after an electrical stunning (*e.g.* Bager *et al.,* 1992). The present work was carried out for assessing the minimum current in a head-only stunning, which involves unconsciousness in all the ducks. In addition, because of the high current magnitudes that were used in the present study, the rate and extent of blood loss were compared after an efficient and a non efficient stun.

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Materials and Methods

Welfare experiment: forty six male force-fed mule ducks were implanted with ECoG electrodes whilst under isoflurane anaesthesia. After they were allowed at least 2 hours to recover before the experiments, the animals were immobilised and hung head downwards in a shackle isolated from earth. The electrocorticograms were recorded before and after the electrical shock, the ECoG recording equipment being isolated during the stunning current delivery. Progressive current strengths were administered at 50 Hz for 4 seconds via two 1-cm diameter steel electrodes fixed on a scissors-type tong. The currents delivered by a constant current stunner were 100 mA (4 birds), 200 mA (12 birds), 300 mA (13 birds), 400 mA (10 birds) and 600 mA (7 birds). The birds were bled 15 seconds after the end of the stun. The ECoG were transformed in frequency power spectra using the Fast Fourier Transforms (FFT), and the evolution of the relative power was calculated in the 2.5-30 Hz frequency band as followed: post-stun power / pre stun power x 100.
Bleeding experiment: fifteen male force-fed mule ducks were bled manually by severing the both carotid arteries, 10 seconds after an electrical head-only stunning. seven ducks received a 300 mA current (insufficient stun) and eight ducks were bled after a 600 mA efficient head-only stunning. The blood amount was measured 10, 20, 40, 60, 80, 100, 120, 140, 160, 180 and 240 seconds after the throat cut, and expressed as percentage of live weight for each bird.

Results and discussion

The applied current magnitudes were progressively increased in order to determine the threshold above which all the animals would be rendered unconscious by the stun. A bird is considered to be unconscious when the relative power of the total frequency band decreases below 10 % of the pre-stun level (Lukatch *et al.*, 1997). The results of the stun efficiency for the various current magnitudes are presented in table 1. Overall, the proportion of birds which were effectively stunned increased with the current intensity, except for the 400 mA treatment. The regain of consciousness, characterised by a brief increase in the relative power above the 10 % level, was observed for some birds towards 40, 50, 60 and 60 seconds post-stun for the 100, 200, 300 and 400 mA treatments, respectively. Ducks' bleeding without prior stunning led to unconsciousness in a mean time from 50 to 70 seconds, depending on animals and bleeding quality (unpublished data). Gregory and Wotton (1986) also observed loss of spontaneous activity in ducks 52 seconds (± 9 SD) after bilateral carotid arteries plus jugular veins cutting. The regain of consciousness observed in the 40-70 seconds post-

stun probably results from a too short-lasting stun efficiency compared to the bleeding mechanisms which lead to insensibility. An efficient current should therefore induce unconsciousness for more than 70 seconds after the end of the stun.

Table 1. Stun efficiency in force-fed ducks after a head-only stunning of various magnitudes.

Current magnitude	Number of birds per treatment	Efficient stun	Inefficient stun	
			From the end of the stun	Regain of consciousness
100 mA	4	1	3	-
200 mA	12	7	1	4
300 mA	13	9	2	2
400 mA	10	6	2	2
600 mA	7	7	-	-

Whereas the duration of the stun-efficiency increased from 40 to 60 seconds, for currents of 100 and 400 mA, respectively, no regain of sensibility was observed for the 600 mA current applications. The latter current magnitude prevent the return of consciousness before EEG extinction occurred from bleeding. According to Gregory and Wotton (1991), an efficient head-only stunning in turkeys is obtained with a 400 mA current. However, in the present study, 2 birds were not rendered unconscious by the stun and 2 others regained consciousness from 60 seconds post-stun with this current magnitude. This would suggest that for a given current intensity, ducks are more difficult to stun than turkeys.

The head-only stunning plus bleeding involved wing flapping and/or convulsions of various intensity for about 30 to 40 seconds after the end of the stun. Severe wing flapping were observed in birds bled without prior stunning (unpublished data), and the presence of these events would characterise an inadequate stun. The convulsions, which were intense and unceasing until 30 seconds after the end of the stun in the 100 mA treatment, decreased in force and continuity with the increasing current magnitude. The 600 mA head-only stunning resulted in very light and spaced out convulsions, concomitant with an efficient stun.

The bleeding kinetics following the 300 and 600 mA head-only stunning are presented in figure 1, the relative blood weight being expressed as percentage of live weights. There was no significant difference in the initial rate of blood loss between the treatments, and 95.8 and 93.6 % of total blood loss were collected 120 seconds after the neck cut for the 300 and 600 mA treatment, respectively. However, after an almost full bleeding (from 160 seconds), the extent of blood loss was significantly higher after a 600 mA than a 300 mA stun. If the current magnitude does not seem to influence the initial rate of bleeding, larger amounts of blood are collected when the ducks are effectively stunned.

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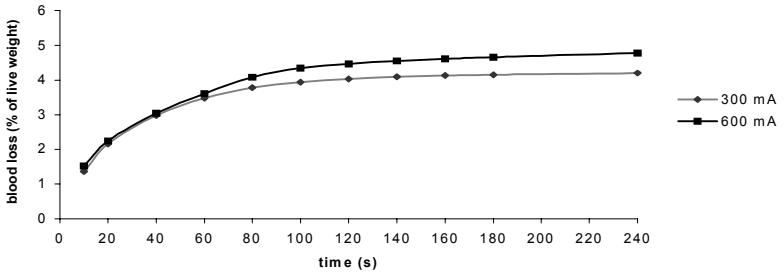
Figure 1. Effect on a 300 and a 600 mA current delivery on the bleeding parameters of seven and eight force-fed ducks, respectively.



Ali *et al.* (1995) also observed that lower applied voltages on chickens in a water-bath decreased bleeding efficiency due to wing flapping and escaping tries.

Conclusion

Whereas some birds regained consciousness after a 400 mA head-only stunning, the stun appeared to be efficient with a 600 mA applied current. The required current for an efficient head-only electrical stunning lies therefore between this two current magnitudes, and a 600 mA stunning would ensure force-fed ducks welfare during slaughtering. Other studies should however be undertaken to precise the lower efficient current associated with good product quality. Indeed, because increasing current magnitudes reduced markedly the force of the convulsions during the clonic phase, the impact of the applied current on product downgrading should be studied at various efficient magnitudes. Moreover, the bleeding experiment showed that whereas the current magnitude had no effect on the initial rate of blood loss, a higher current delivery increased bleeding efficiency.

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COMPARISON OF THREE GAS STUNNING SYSTEMS FOR TURKEYS ON ANIMAL WELFARE, CARCASS DEFECTS AND MEAT QUALITIES.

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Keywords: turkey, gas stunning, animal welfare, meat quality

Abstract

Electrical stunning in a water bath is the most commonly used stunning method under commercial conditions. Turkey manufacturers are very interested in an alternative stunning method using controlled atmosphere. Gas stunning has two major advantages: it improves working conditions at the shackling point because the birds would be immobilised and inert, it reduces the stress of animals by avoiding an upside down position. Consequently, the frequency of carcass defects should be reduced. Stunning systems were assessed for animal welfare during anaesthesia induction, bleeding efficiency and carcass and meat qualities.
Gas mixtures were : 1- a biphasic system (1 min CO_2 / O_2 ; 2 min CO_2), 2 - a biphasic system (1 min CO_2 / O_2 ; 2 min CO_2 / O_2), 3 – nitrogen atmosphere (O_2< 2%3 min).
In our experimental conditions, the stunning method with oxygen gave the best results in terms of animal welfare, bleeding efficiency and carcass and meat quality.

Résumé

Les industriels du secteur volaille sont demandeurs d'un procédé d'étourdissement alternatif à l'électronarcose, méthode généralement utilisée en France. Cette méthode d'anesthésie gazeuse présente deux intérêts majeurs 1- une amélioration des conditions de travail pour le personnel à l'accrochage puisque les animaux sont manipulés inertes 2- une réduction du stress des animaux car ils ne sont plus suspendus la tête en bas. Notre étude consiste à évaluer l'influence des mélanges gazeux susceptibles d'être mis en oeuvre dans les abattoirs de dinde sur le comportement des animaux pendant les phases d'inhalation des atmosphères gazeuses, l'efficacité de la saignée et la qualité de la carcasse et de la viande. Trois méthodes d'étourdissement au gaz ont été

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comparées : 1 et 2 : les animaux sont placés successivement dans deux atmosphères enrichies en dioxyde de carbone et/ou en oxygène ; . méthode 3, les animaux étaient exposés à une atmosphère appauvrie en oxygène. Dans nos conditions expérimentales, les méthodes reposant sur un enrichissement de l'atmosphère en CO_2 et O_2 se sont révélées supérieures en termes de bien être, d'efficacité de saignée et de qualité des carcasses et des viandes.

Introduction

Electrical stunning in a water bath is the most commonly used stunning method under commercial conditions. Turkey manufacturers are very interested in an alternative stunning method using controlled atmosphere. Gas stunning has two major advantages: it improves working conditions at the shackling point because the birds would be immobilised and inert, it reduce the stress of animals by avoiding an upside down position. Most of stunning studies which have been conducted on chicken showed that the efficiency of gas stunning depends largely on the composition in gas. Similar results were reported for the carcass quality traits. The effect of gas stunning on meat quality remains unknown (Raj et Tserveni-Gousi 2000 ; Fernandez *et al.*, 1998). This study was carried out to assess different gas mixtures which could be implemented in turkey slaughter plants. We focused our work on turkey observation during stunning and until bleeding, and on carcass and meat qualities.

Material and methods

Three gas mixtures were compared : 1- a biphasic system (1 min 40 %CO_2 / 30 % O_2 ; 2 min 80 % CO_2), 2 - a biphasic system (1 min 40 % CO_2 / 30 % O_2 ; 2 min 55 % CO_2 / 30 % O_2), 3 – nitrogen atmosphere (O_2< 2% 3 min). Two boxes were built with plexiglass sides for the observation of animals.
A total of 54 toms (12 week old) were stunned (18 turkeys/treatment) after being weighed. The experiment was repeated 3 times and the stunning methods were balanced for each trial. The gas mixture was under control (gas analysor, Abyss). The turkeys were introduced into the box and pure gas were added to keep the gas concentration stable (Table 1). For biphasic systems, the animal were rapidly transferred from the first box to the second one. For nitrogen system (method 3), when the turkey was put in the box, the amount of oxygen rose slightly. The turkey behaviour was observed during stunning and bleeding. Whatever the method, after 3 minutes, a bilateral section of carotid arteries and jugular veins was performed. Blood

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pH and partial pressure of CO_2 and O_2 were measured (IRMA, Diametrics Medical, Angleterre). The rate and the amount of blood loss were measured every 20 s during 3 minutes and expressed as % of live weight (blood density = 1.04). Muscle *Pectoralis superficialis* pH was measured 20 minutes and 24 h *post mortem*. Carcass defects were checked the day after slaughter (engorged wing veins, haemorrhages, hip and shoulder dislocated/broken joint..). The right breast muscle was cut in scallops on which colour and drip loss were measured. Colour was evaluated by Minolta spectrophotometer (CR 300). Curing and cooking yield was determined on the left breast muscle. The breast was injected with brine (15%) every cm^2, vacuum-packed and diffusion of the brine took place over night. Cooking in a water-bath was stopped when internal temperature rose 68 °C.
For numerical data, analyses of variance were performed using the GLM (General Linear Model) procedure of SAS. The model included the main effect of stunning method, day of slaughter and their interaction. When variance analysis revealed a significant effect, differences between groups were tested using Duncan multiple rang test. Carcass defect score data were treated by non parametric analysis of variance using the NPAR1WAY procedure of SAS. Means were compared using the Wilcoxon non parametric test.

Results

When the turkey was put in the box, we observed a loss of posture occurring after 30 s on average (table 2). All the turkeys showed intense gasping. Moreover, the head of the animal fell backward, stretching its neck until putting the head on its back. There was no significant difference between the 3 methods. When wing flapping occurred, it was after 2 minutes. No wing flapping was noticed with the method 2. With the method 1, 2 turkeys showed intense wing flapping. But with the method 3, almost all the turkeys had intense wing flapping, so vigorous that half of the turkeys exhibiting wing flapping had broken shoulder or dislocated shoulder joint (figure 2).
With the method 2, the turkeys were stunned and still alive. But the with the method 1 or 3, the turkeys were stunned/killed (100 % and 78 % respectively).
Consequently, the rate of blood loss was less with the method 1 and 3 during the 80 first seconds (Figure 1). The initial rate of blood loss was higher when the animal were not killed. Goksoy *et al*. (1999) showed on electrically stunned broilers that non fibrillated birds had the fastest blood loss rate. But with the 3 methods, the total bleed out remained the same after 3 minutes. Similar results were reported on broiler and turkey by Raj & Gregory (1991) and Raj *et al.* (1994).
The data of blood pH and blood gas are presented in table 3. The turkeys stunned with method 1 and 2 exhibited lower blood pH and higher pCO_2 and HCO_3^-. The results

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indicated a metabolic acidosis which had no effect on *post mortem* muscle metabolism as we will see below.
Carcass defects are shown on figure 2. Turkeys stunned with the method 3 (nitrogen) had numerous carcass defects : at least 44% of the carcass had a broken wing or dislocated joint and more than 50% had engorged wing veins and/or haemorrhages in legs. This results can be explained mainly by the vigorous wing flapping which occurred during stunning.
Muscle pH, colour development and cooking yield are presented in table 4. Initial pH differed with the stunning methods. Higher pH was found with the method 1. With this method, the turkey were stunned/killed and there was no wing flapping during stunning (method 3) nor during bleeding (method 2). Turkeys stunned with method 1 had the lowest ultimate pH. Curing and cooking yield was higher in breast muscle from birds stunned with the method 1. The stunning method did not affect the lightness (L*) nor the yellowness (b*) of the muscles. But redness (a*) was higher on breast meat of turkey stunned with method 3 whatever *post mortem* time.

Conclusions

The gas stunning methods based on oxygen / carbon dioxide enrichment showed better results regarding animal welfare and carcass and meat quality than the third method based on anoxia. However, our experimental stunning device did not allowed to achieve less than 2 % O_2 immediately after having put in the turkey in the box. The methods need to be evaluated in a slaughter plant and compared to electrical stunning which is the common commercially used method .

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Thanks

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Table 1. Gas concentration for the gas stunning methods

Method	**1**		**2**		**3**
Stage	1	2	1	2	-
Gas	CO_2/O_2	CO_2	CO_2/O_2	CO_2/O_2	O_2/N_2
Theorical concentration %	40/30	80	40/30	55/30	2/98
measured concentration (1)	40.8/30.7	80.1	40.4/30.5	55.5/30.5	(2)
time	1 min	2 min	1 min	2 min	3 min

(1) mean of measured CO_2 and O_2 partial pressures. The complementary gas is N_2
(2) partial pressure of O_2 was 6.7 % after 30 s, 3.4 % after 2 min and 2,0 % 3 min after the turkey was introduced in the box.

Table 2. Behaviour of turkey during gas stunning

Method	Loss of posture (sec)	Number of turkeys showing intense wing flapping	Number of dead turkeys after 3 min gas stunning
Method 1 (CO_2/N_2)	27 ± 7	2 / 18 [a]	18 / 18 [a]
Method 2 (CO_2/N_2)	26 ± 7	0 / 18 [a]	0 / 18 [b]
Method 3 (N_2)	33 ± 10	16 / 18 [b]	14 /18 [a]

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Table 3. Blood pH and bicarbonates measurements, CO_2 and O_2 partial pressures

Method	Blood pH	PCO_2 mmHg	pO_2 mmHg	HCO_3 mM
Method 1(CO_2/N_2)	6.91 ± 0.07	173 ± 23	48 ± 13	35 ± 2
Method 2 (CO_2/N_2)	7.07 ± 0.07	93 ± 23	76 ± 7	26 ± 2
Method 3 (N_2)	7.25 ± 0.14	42 ± 14	33 ± 18	17 ± 2

Table 4. Effect of stunning method on muscle pH, colour development and cooking yield

		Method 1 (CO_2/N_2)	Method 2 (CO_2/N_2)	Method 3 (N_2)	p
pH 20 min		6.45a	6.25b	6.21c	***
pH 24 h		5.69a	5.74b	5.72a	*
J+1	L*	51.7	52.1	51.7	ns
	a*	3.9^a	4.3^a	5.3^b	***
	b*	2.3	2.1	2.1	ns
J+4	L*	51.8	52.3	51.6	ns
	a*	3.1^a	3.8^b	4.7^c	***
	b*	4.9	4.6	4.9	ns
J+8	L*	52.1	52.9	51.2	ns
	a*	2.5^a	3.1^a	4.0^b	***
	b*	5.5	5.8	5.3	ns
Cooking yield %		89.1^a	87.4^{ab}	86.5^b	*

Figure 1 : Effect of stunning method on bleeding efficiency

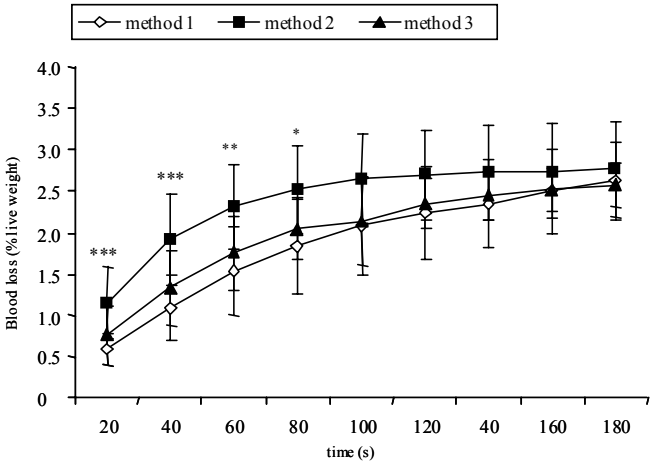

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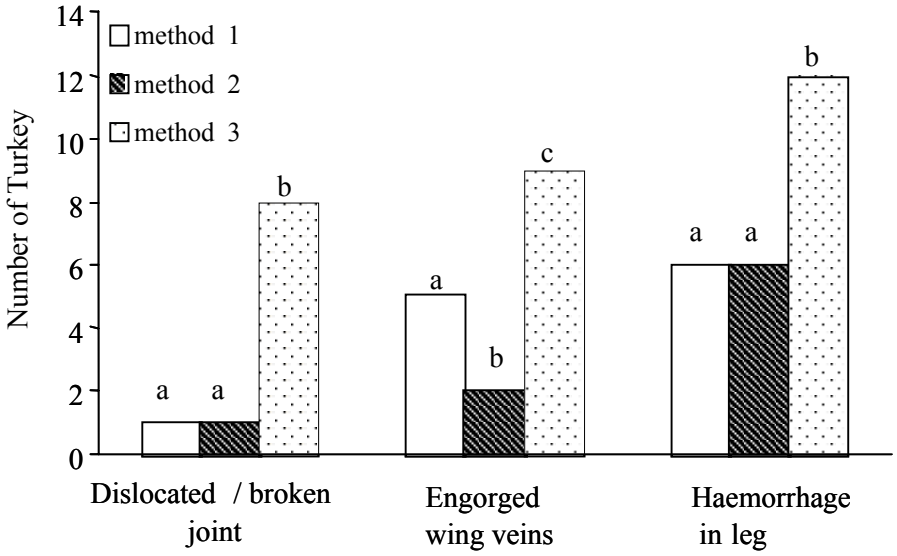
Figure 2 :Effect of gas stunning method on carcass quality defects in turkey



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A SURVEY ON POULTRY SLAUGHTERHOUSES IN TURKEY: INCIDENCE OF CARCASS DEFECTS AND MEAT QUALITY RELATED TO STUNNING VOLTAGE

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Keywords : broiler, carcass defects, meat color, stunning voltage

Abstract

This study was done to evaluate carcass defects and breast meat color and pH of broilers stunned at different voltages prior to slaughter. Six slaughterhouses were visited and carcass defects were determined on 300 male broilers (50 broilers/each slaughterhouse) after chilling. Furthermore, nine breast muscles were randomly selected from each slaughterhouse and ultimate pH and meat color were measured. Although slaughterhouses had the same technology, stunning voltage varied between the plants. The statistical analysis showed that the incidences of red wing tips, red feather tracts and breast muscle hemorrhages were influenced by the stunning voltage. In addition, high stunning voltage (>65V) increased pH and redness.

Résumé

Cette étude a été réalisée dans le but d'évaluer les défauts de carcasses, la couleur et le pH du muscle pectoral de poulets étourdis avec des courants de tension variable. Six abattoirs ont été visités et les défauts de carcasse ont été enregistrés sur 300 poulets mâles (50 / abattoir) après réfrigération. Neuf muscles pectoraux / abattoir ont été choisis au hasard pour les mesures de pH et de couleur. Les six abattoirs présentaient des technologies d'abattage en tous points identiques, à l'exception de la tension du courant utilisé lors de l'électronarcose en bain électrifié. La tension du courant influençait significativement l'incidence des défauts suivants : extrémité des ailes rouges, sicots rouges, hématomes dans le muscle pectoral. Les courants de plus de 65 V entraînaient une augmentation du pH et de l'indice de rouge des muscles.

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Introduction

Although preslaughter stunning has been universally used in poultry industry for many years to immobilize poultry prior to slaughter, electrical stunning conditions are not standardized in terms of current, duration and frequency. Various electrical parameters have been reported to have different effects on carcass defects and on the rate of post-mortem glycolysis (Raj *et al*., 1990; Heath *et al*., 1994; Hillebrand *et al*., 1996; Sante *et al*., 2000). Haemorrhage on wings, red wing tips, engorgement of wing veins, breast muscle haemorrhage and broken or dislocated bones have been attributed to stunning voltage (Bilgili, 1992; 1999; Gregory and Wilkens, 1989). Several authors showed that high voltages (150V) result in more carcass defects whereas lower voltages (60 to 65V) result in rigor-slowing effects. (Sams, 1999; Gregory and Wilkins, 1989) Ozkan *et al*. (2001) observed an increament in the severity of red wing tips when stunning voltage increased from 50 to 100V.

The effect of stunning on meat quality was reported by Papinaho and Fletcher (1995) who indicated that stunning amperages between 0 and 200 mA had effects on the early rate of rigor development but there were no consistent effects on final breast meat quality. Craig and Fletcher (1997) reported that stunning with a 125mA, 50 Hz constant AC for 5 s reduced initial blood loss and delayed early rigor development compared to the 10.5V, 500 Hz pulsed DC for 10s stunning treatment.

Stunning duration also affects quality characteristics. As stunning time increased from 0 to 10s pH and yellowness increased while other color values were not affected by stunning duration (Young and Buhr, 1997).

The increase in quality defects results in substantial economic losses in broiler industry. As the poultry industry has become more involved in further processing, the importance of not only carcass quality such as absence of defects but also meat quality such as ante-mortem pH levels and color is increased. In Turkey, where electrical stunning voltage varies among poultry plants, the incidence of stunning related defects appeares to be high. This study was done to evaluate carcass defects and breast meat color and pH of broilers stunned at different voltages prior to slaughter.

Material and Methods

The experiment was carried out under commercial conditions.

Animals: All broilers used in this study were from the same commercial broiler genotype. Broilers were reared in floor pens under typical commercial conditions. Feed and water were supplied for *ad libitum* consumption and 23L:1D lighting was

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applied. The birds were subjected to a 8-12 h of feed withdrawal period prior to slaughter. Water was provided for *ad libitum* consumption until catching and crating. The duration of transportation from farm to slaughterhouse was 1 h.

On each of the six commercial poultry slaughter houses, 50 male broilers (total 300 broilers) were investigated. The average slaughter weight was 2050 g and carcass weight was 1500 g.

Slaughter procedure: Birds were processed using on-line procedures. The technology was the same among the six slaughterhouses except for the voltage and duration of electrical stunning in a water bath. The stunning voltage (alternating current) and duration were 1) < 49 V and 11 s, 2) between 50 and 64 V and 4s, and 3) >65V and 11s. The current frequency was 50 Hz. There were two slaughterhouses for each stunning group.

Carcass defects: Carcasses were weighed after chilling. To minimize the effect of variation in bird size, extremes in carcass weights were excluded to obtain 1500 g with a standard deviation of ± 50g. One person subjectively evaluated carcass defects on each carcass. The following defects were recorded: hemorrhagic wing veins, red wingtips, hemorrhage in breast and thight, broken breast bones, red feather tracts. The defects were scored as presence or absence and the results were expressed as % of carcasses showing the defect (to be confirmed by the authors).

Meat quality: The measurement was taken on the external surface of the muscle away from feather tract marks. The left breast muscle was harvested immediately after chilling and placed in labeled plastic bags , aged on ice until 24 h *post mortem*. The pH values were measured by inserting a probe (pH meter, Hanna instrument model 8314) into the muscle. Colour coordinates (L*, a*, and b*) were measured using a portable Minolta CM 508d spectrocolorimeter.

Statistical analysis: Chi-square analysis was performed using SAS (SAS Inst., 1989) to test effect of stunning on carcass defects. Analysis of variance was used to test the influence of stunning voltage on meat quality. When a significant effect was noted means were compared by Duncan's multiple-range test.

Results and discussion

The incidence of red wing tips was similar when birds were stunned at < 49V , >50V 11 s or 4 s. The >65 V resulted in an increase in red wing tips. Although the incidence of wing vein hemaorrhages increased in birds subjected to the >65V stunning, it did not differ significantly between the birds subjected to the <49V and 50-64V stunning voltages (Table 1).

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There was significant differences among stunning voltage for red feather tracts observed on wing, breast and leg; the incidence was the lowest for the birds stunned at <49V.
Breast muscle haemorrhage incidence was increased with increasing stunning voltage, while the incidence of hemorrhages on thight was similar among the groups.
Although it was reported that stunning with 50 Hz resulted in broken bones (Gregory *et al*., 1990), only one broken breast bone was observed here (data not shown). This result is in agreement with Ozkan *et al.(*2001).

Table 1. Influence of stunning voltage on carcass defects (number of broilers with carcass defect was given in parenthesis for each stunning voltage)

	<49 V 11s	50-64V 4s	>65V 11s	χ^2
Wing				
Red wing tips	12.7 (34)	15.7 (42)	71.6 (192)	29.8**
Wing vein hemorrhage	20.5 (27)	15.9 (21)	63.6 (84)	2.4
Red feather tract	2.4 (3)	14.2 (18)	83.5 (106)	44.2**
Breast				
Red feather tract	8.3 (7)	11.9 (10)	79.8 (67)	10.1**
Breast muscle hemorrhage	4.5 (2)	4.5 (2)	90.9 (40)	16.4**
Leg				
Red feather tract	10.4 (7)	11.9 (8)	77.6 (52)	4.9*
Hemorrhage in thight	23.7 (14)	20.3 (12)	55.9 (33)	3.9

*p<0.05, **p<0.01

The results for raw breast meat pH and color are presented in Table 2. Stunning voltage >65 V significantly increased lightness and redness which averaged 50.9 and 0.10, respectively whereas the <49V treatment resulted in the lowest lightness value (L=46.3). Stuning voltage above 65V increased yellowness of breast meat. The pH value of breast muscle tended to increased (p= 0.09) when broilers were stunned with currents above 49V.

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Table 2. Influence of stunning voltage on breast meat pH and color (24h)

	<49 V	50-64V	>65V	Significance (P value)
Lightness, L*	46.29 [b]	47.74 [b]	50.89 [a]	<0.001
Redness, a*	-1.63 [b]	-2.04 [b]	0.10 [a]	0.006
Yellowness, b*	2.61 [a]	2.51 [a]	1.53 [b]	0.052
Ratio a*/b*	-0.69	-0.92	-0.25	0.815
pH	6.02	6.11	6.11	0.088

In conclusion, the present study confirmed that the incidences of red wing tips, red feather tracts and breast muscle hemorrhage were influenced by the stunning voltage. The experiment was carried out under commercial conditions. The housing, catching and transportation were very similar among flocks. Therefore, these differences in carcass quality can be reasonably attributed to the stunning voltage. Barbut (1997) reported that breast muscle samples with L* > 49 would be classified as PSE meat. On this basis, we can conclude that higher stunning voltage (>65 V) would induce PSE meat in this study.

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This classification is non exhaustive as it is based on key words provided by the authors and do not included those present in the abstract or text

XVIth European Symposium on the Quality of Poultry Meat
&
Xth European Symposium on the Quality of Eggs and Egg Products

September 23rd – 26th, 2003

Saint-Brieuc
France

Proceedings

Volume III : Quality of Eggs and Egg Products

Volume I : New European regulations and their impact - Hygiene and microbiology of poultry production - Evaluation and control of risks in poultry production
Volume II : Quality of Poultry Meat
Volume III : Quality of Eggs and Egg Products

Realisation of proceedings : Geneviève Clément and Marie-Pierre Rouxel, ISPAIA

Introduction

This volume contains all the manuscripts of the reviews, oral communications and posters presented during the 10th European symposium on the quality of Eggs and Egg products held the 23-26th of September 2003 in conjunction with the 16th European Symposium on the Quality of Poultry Meat in Saint Brieuc – Ploufragan, France. It is additional to the volume devoted to the Poultry meat quality sessions and to that containing the economical and hygienic quality sessions. The symposia are organized under the auspices of the French branch of the WPSA, the Poultry meat (group 5) and egg quality (group 4) working groups with the collaboration of AFSSA (French Agency for Food Safety), INRA (French Institute for Agronomical Research), ISPAIA (Higher Institute for Animal Production and Food Industries) and ITAVI (Technical Institute for Poultry Production).

France hosted these symposia 10 years ago in Tours. Since Egg producing system have been questioned in Europe because of the welfare issue, being at the origin of a lot of uncertainties for the producers and all the egg industry. Some European country planed to ban totally the cage system without having in hand any alternative housing system acceptable for maintaining high quality standard and safety of eggs. The first demand of the consumer concerns safety. Egg is the only animal protein with self defence system but can be at origin of nutritional toxi-infection because of its consumption as raw product. That should be taken in 2005 by the European veterinary committee in charge of advising for the final decision to be applied in 2012 for Europe as it will largely affect competitiveness of egg production. Content of this volume reflects these European preoccupations by describing furnished cages system or by proposing non invasive evaluation of egg quality to sort eggs. An addition evolution is the development of egg product. The initiatives in this area or to valorise specific components of eggs remains too limited. There are however tremendous possibilities because of the remarkable biological properties of egg constituents. Speakers from different part of the work have been invited to stimulate this field in Europe.

The venue of the symposium in Brittany, the support from governmental and private institutions for offering you social events in warm environment, the quality of the presentation should assure a profitable and enjoyable event in St Brieuc.

Yves Nys
INRA-WPSA

Introduction

Ce tome 3 contient l'ensemble des manuscrits des revues, communications orales et affiches présentées les 23-26 septembre 2003 au cours du 10ème congrès Européen sur la qualité des œufs et des ovo-produits, ainsi qu'au 16éme congrès Européen, conduit parallèlement sur la qualité de la viande de volaille et qui ont eu lieu à St Brieuc-Ploufragan, France. Ce tome est complémentaire au volume 2 consacré aux sessions sur la qualité de la viande et de celles concernant les aspects économiques et hygiénique des produits avicoles.

Ces deux congrès ont été organisés par la branche française de la WPSA, les groupes de travail européens, qualité de la viande de volaille (5) et qualité de l'œuf (4) avec l'aide de l'AFSSA (Agence Française pour la Sécurité Alimentaire), INRA (Institut National de la Recherche Agronomique), l'ISPAIA (Institut supérieur de la Production Animale et de l'Industrie Alimentaire) et l'ITAVI (Institut Technique de l'Aviculture).

La France a hébergé ces congrès sur la qualité des produits avicoles 10 ans auparavant. Depuis, le système de production d'œuf en cage a été remis en question en Europe avec la prise en compte du bien-être des poules. Ceci a déstabilisé les producteurs et firmes services. Certains pays européen envisageant même d'abandonner totalement le système de production d'œufs en cages, sans avoir en main de systèmes alternatifs assurant une qualité de l'œuf, notamment hygiénique satisfaisante. Pourtant, la première exigence du consommateur reste la sécurité alimentaire. L'œuf est la seule protéine animale ayant un système de défense contre la contamination bactérienne. Pourtant, il est un des aliments à l'origine de toxi-infection car il est consommé cru. Cet aspect risque devrait être prise en compte par le comité Européen chargé, en 2005, de fournir des propositions pour le règlement sur l'élevage des poules, applicable en 2012. Cette décision sera capitale pour la compétitivité de la production Européenne dans le monde. Le contenu de ce volume 3 traduit ces nouvelles préoccupations en proposant des systèmes alternatifs de cages aménagées, des systèmes non invasif de mesure de la qualité des œufs pour les trier. L'augmentation de la part des ovo-produits est également une nouvelle composante de la production d'œufs. Les initiatives pour proposer de nouveaux produits restent trop limitées. Il existe pourtant un potentiel considérable d'une utilisation de constituants de l'œuf qui présentent des propriétés biologiques remarquables. Nous avons invité des orateurs pour stimuler ce champ d'activité.

L'organisation de ces congrès en Bretagne, l'aide de sponsors publics et privés favorisant la mise en place d'un programme social dans un environnement chaleureux, la qualité des communications devraient vous assurer un séjour agréable et informatif à St Brieuc.

Yves Nys
INRA - WPSA.

Proceedings edited by :
Yves Nys (INRA)

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Formation of egg and chemical competition

CLUSTERIN IS AN EGG WHITE AND EGG SHELL PROTEIN

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Keywords : Clusterin, Avian egg, Shell, White, Chaperone

Abstract

The present report identifies chicken clusterin as a component of the eggshell and egg white. This extracellular clusterin originates in the uterine fluid, where it is present as a disulfide-bonded heterodimer. Clusterin mRNA was identified in the magnum and uterine segments of the oviduct. Western blotting indicated major clusterin production in the magnum. In addition, clusterin was also detected in egg white by Western blotting. In the decalcified eggshell, immunofluorescence and colloidal-gold immunocytochemistry revealed that clusterin was predominantly localized in the palisade and mammillary layers, but also in the shell membranes. Clusterin could function in the uterine fluid to prevent premature aggregation and precipitation of eggshell matrix components before and during their assembly into the rigid protein scaffold necessary for ordered mineralization.

Résumé

Cet article décrit l'identification de la clusterine aviaire dans la coquille et le blanc d'œuf. La clusterine est extracellulaire dans le fluide utérin et sous forme d'un hétéro dimère lié par un pont disulfure. Le messager de la clusterine a été identifié dans le magnum et l'utérus. Le western blot indique que la clusterine est majoritairement produite dans le magnum. Cette même technique a également montré que la clusterine est une protéine du blanc d'œuf. L'immunofluorescence et l'immunohistochimie de la coquille décalcifiée montre que la clusterine est principalement présente dans la

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couche mammillaire et palissadique, mais aussi dans les membranes coquillères. La clusterine pourrait agir dans le fluide utérin en empêchant l'agrégation prématurée et la précipitation des composants de la matrice organique avant et après leur assemblage en protéines rigides nécessaire à la minéralisation ordonnée de la coquille.

Introduction

The identification and characterisation of egg proteins are important prerequisites for understanding the natural defences of the egg. The shell constitutes a natural physical barrier against bacteria; eggshell matrix proteins are crucial in determining the mechanical properties of the shell. In the egg white, the growth of contaminating bacteria are limited by the bacteriostatic properties of proteins such as lysozyme and ovotransferrin. The aim of the present report is to show for the first time that the presumptive extracellular chaperone clusterin is a component of the eggshell matrix and of the egg white, and that the α/β heterodimer described in mammalian tissues and fluids also exists in the chicken.

Material and Methods

Clusterin was purified from eggshell using a previously described procedure (Mann 1999; Mann and Siedler 1999). The soluble fraction was re-dissolved in 1% trifluoroacetic acid containing 8 M guanidinium hydrochloride and fractionated using a reversed phase column. Clusterin fractions were reduced, pyridylethylated (Mann et al., 1999) and cleaved with CNBr. The peptides were then separated by size exclusion chromatography and by reversed phase chromatography with a C18 column (Mann et al., 1999). Sequence analysis was performed on an Applied Biosystems sequencer.
Eggshell matrix proteins and tissue samples were extracted as described by Gautron et al. 1997; 2001. Proteins were separated by SDS-PAGE on 10% or 12.5% gels, blotted onto polyvinylidene difluoride (PVDF) membranes, and clusterin was identified by Western blotting following established protocols (Gautron et al., 2001). We used a rabbit polyclonal anti-clusterin antibody (diluted 1:20,000) directed against residues 394 - 410 of the protein (Mahon et al., 1999), which is specific for the β subunit of the α/β heterodimer. The enhanced chemiluminescence method was used to detect immunoreactive bands.
Immunolocalization of clusterin was detected by immunofluorescence as described (Hincke et al. 1995). In addition, the high-resolution, colloidal-gold

immunocytochemical technique (McKee and Nanci, 1995) was used to localize clusterin at the ultrastructural level.
Total RNA was prepared from different parts of the oviduct (magnum, white and red isthmus, uterus) and from liver, kidney and duodenum. Reverse transcription was performed with 5 µg RNA using random primers and 200 U superscript II reverse transcriptase (Rnase H^-, Life Technologies) at 42°C for 60 min. Specific PCR primers were designed to amplify chicken clusterin cDNA. Northern blotting was performed using standard techniques (Sambrock et al., 2000). A 653 bp PCR product was radiolabeled with 32P and used as a cDNA probe for Northern blotting.

Results and Discussion

During purification of OC-17 from chicken eggshell (Mann 1999; Mann and Siedler, 1999), a protein of 70 kDa in SDS-PAGE was detected. This shifted to 35 kDa under reducing conditions, indicating that it consisted of two disulfide-bonded units. The protein was further purified, and N-terminal sequence analysis yielded a mixture of two amino acid sequences in exactly equal proportions indicating that the protein was a heterodimer. The protein was cleaved and the peptide fragments were identified. The fragments matched perfectly the published sequence of chicken clusterin (Mahon et al., 1999). Detailed analysis of the fragments demonstrated that eggshell clusterin consists of the α/β heterodimer which is commonly encountered in mammals, but which has not yet been observed in the chicken. No free subunits of clusterin were encountered during the sequence analysis of eggshell extracts - only the clusterin heterodimer was observed.
An anti-serum was used to localise clusterin in the eggshell. Immunofluorescence and immunogold labeling showed that all calcified regions of the shell and the membranes displayed immunostaining, but clusterin was predominantly localised in the palisade and mammillary layers, and in the mantle and core of the eggshell membranes. The tissue origin of clusterin was investigated by Western blotting. Clusterin was strongly detected in magnum where egg white proteins are secreted. Only a faint band after a long exposure time was obtained in the uterus where eggshell mineralization takes place. A novel finding of the present report was the presence of an immunoreactive band in the egg white, and the subsequent identification of clusterin as an egg white protein. These results are consistent with those obtained by RT-PCR and Northern blotting techniques. Different oviduct segments and a number of non-reproductive tissues were analyzed (Figure 1 and 2). RT-PCR revealed that clusterin expression levels were highest in magnum, uterus, brain, spleen and bursa of Fabricius, moderate in white and red isthmus and kidney, and low or variably detectable in liver and duodenum (Figure 1). Relative clusterin expression was further investigated and

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quantified by Northern blotting (Figure 2), which revealed high levels of the 1.6 kb message only in magnum and uterus of those tissues tested (significantly different, $p < 0.001$ for each of these compared to the other tissues).

Studies with clusterin have demonstrated that it exerts a powerful chaperone-like activity with egg white ovotransferrin and lysozyme, as well as with other purified proteins (Poon et al., 2000; Lakins et al., 2002; Poon et al., 2002a; Poon et al., 2002b). These studies reveal that clusterin binds to and stabilizes slowly aggregating proteins. Clusterin prevents the slow aggregation of proteins that leads to irreversible precipitation by preferentially recognizing partially folded protein conformations that are responsible for this process. Clusterin therefore appears to function extracellularly like the family of small heat-shock proteins (sHSP's) that stabilize intracellular proteins under stress conditions (Haslbeck, 2002). Lysozyme and ovotransferrin are major components of the egg white (12% and 3.4% of the total protein, respectively (Burley et al., 1989); the clusterin which we have documented in the egg white could stabilize these (and other) proteins during incubation of the developing avian embryo (3 weeks at 40^{o}C).

Lysozyme and ovotransferrin are also constituents of the eggshell matrix (Hincke et al., 2000; Gautron et al., 2001). The uterine synthesis and secretion of clusterin into uterine fluid, from which it becomes concentrated in the calcifying shell, suggests that clusterin could function as a chaperone to prevent the premature aggregation and precipitation of stressed, partially unfolded or sticky proteins before and during their assembly into the eggshell organic matrix. Thus clusterin may regulate the assembly of eggshell matrix components into the protein scaffold associated with ordered mineralization.

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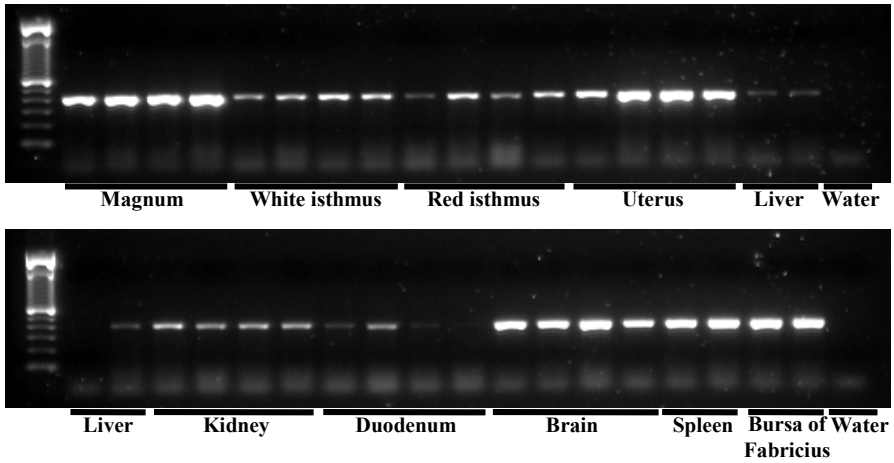

Figure 1. RT-PCR to detect expression of clusterin mRNA in chicken tissues.
Total RNA was extracted from the different parts of the oviduct and from other tissues. Tissues were harvested from ISA brown laying hens, except spleen and Bursa of Fabricius (white leghorn hens). Following reverse transcription, PCR was performed using Clusterin primers for amplification of 397 bp product. Standards (100bp ladder) are on the left

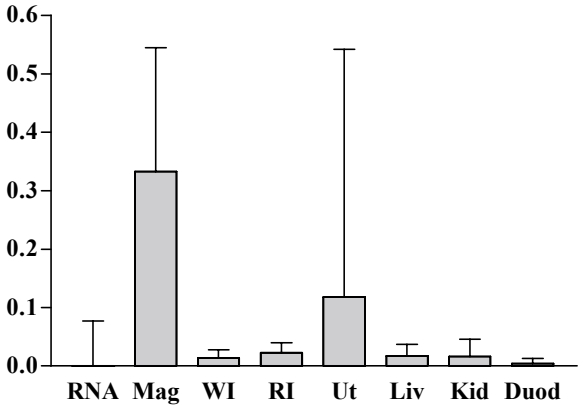

Figure 2: Northern blotting to detect clusterin mRNA. Total RNA was extracted from segments of oviduct (magnum (Mag), white isthmus (WI), red isthmus (RI) and uterus (Ut)) and other hen tissues (liver (Liv), kidney (Kid), duodenum (Duod). Relative abundance (arbitrary units) of clusterin message was determined. Expression was the highest in magnum and uterus. Levels in all other tissues were negligible.

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GENE ORGANIZATION OF OVOCALYXIN-32, AN EGGSHELL MATRIX PROTEIN.

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Keywords : eggshell, ovocalyxin, latexin, mineralization, calcite

Abstract

The avian eggshell is one of the fastest calcifying processes known. Characterization of individual matrix components of the shell is necessary in order to determine their influence upon calcite crystal shape, size and orientation during eggshell calcification. To this end, we have identified a novel 32-kDa protein, ovocalyxin-32 (OCX-32). OCX-32 possesses limited identity (about 30%) to two different mammalian proteins: latexin, a carboxypeptidase A (CPA) inhibitor, and RAR-TIG1, a skin protein encoded by a retinoic acid receptor (RAR) - responsive gene. In order to gain insight into the function of OCX-32 during eggshell calcification, we have compared the sequences of OCX-3 with human and mouse latexin and RAR-TIG1. In spite of low levels of sequence identity, an alignment of the protein sequences reveals that their genes have a virtually identical organization with respect to exon boundaries. This result suggests that there is an evolutionary relationship between these genes. Studies with recombinant OCX-32 will provide further insight into its activity and role during the termination of mineral deposition in the eggshell. (supported by NSERC, Ontario Egg Board and the Poultry Industry Council).

Résumé

La formation de la coquille de l'œuf est un des plus rapides processus de minéralisation du monde vivant. La caractérisation des composants individuels de la matrice organique de la coquille, est nécessaire pour déterminer son influence sur la forme, la taille et l'orientation des cristaux de calcite pendant la calcification de la coquille. Dans ce but, nous avons identifié une nouvelle protéine de 32 kDa, l'ovocalyxin-32 (OCX-32). L'OCX-32 a une identité limitée (environ 30 %) avec 2 protéines sans relations fonctionnelles a priori. Il s'agit de la latexin, un inhibiteur de

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la carboxypetidase A, et de RAR-TIG1, une protéine de la peau synthétisée à partir d'un gène contenant une séquence liant le récepteur de l'acide retinoique. Nous avons comparé ces 2 séquences de manière à mieux comprendre la fonction de l'OCX-32 pendant la calcification de la coquille. Malgré les faibles niveaux d'identité de ces 2 séquences, un alignement de ces séquences protéiques révèle que les frontières exon-exon ont une organisation pratiquement identique entre ces 3 protéines. Il pourrait donc exister une relation dans l'évolution des gènes respectifs de ces protéines. Des études complémentaires seront faites avec de l'OCX-32 recombinante afin de préciser son activité et son rôle lors du dépôt minéral de la coquille et notamment lors de la phase terminale (travail financé par NSERC, Ontario Egg Board and the Poultry Industry Council).

Introduction

The protein components of biominerals (matrix proteins) influence the shape and strength of the final structure by modulating crystal nucleation and growth, which in turn defines the textural properties and crystal shape that strongly affect mechanical performance (Berman et al., 1993). The avian eggshell forms in the uterus (shell gland) portion of the oviduct, in an acellular milieu that is supersaturated with respect to calcium and bicarbonate, and which contains a changing variety of protein species at the initial, calcifying and terminal stages of shell formation (Nys et al. 1999). The eggshell possesses a complex mixture of matrix proteins that are differentially distributed between the inner (mammillary) and outer (palisade) layers of the shell due to precise temporal and spatial control of eggshell formation (Hincke et al., 1992; Hincke et al., 1995; Hincke et al., 1999; Gautron et al., 2001). Eggshell-specific proteins termed ovocleidins and ovocalyxins have been identified by N-terminal amino acid sequencing and immunochemistry, and cloned. One of these novel chicken eggshell proteins, Ovocalyxin-32 (OCX-32), possesses limited identity (30%) to two apparently unrelated proteins: latexin and RAR-TIG1 (Gautron et al., 2001; Hincke et al., 2003). In order to gain insight into the function of OCX-32 and its potential role during eggshell calcification, we have further investigated the sequence similarities between these proteins and the relative organization of their genes.

Material and Methods

Genomic DNA was extracted from blood collected from white Leghorn hens using the GenElute DNA miniprep kit (Sigma) and used as a template for PCR as per

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established protocols (Hincke et al., 1999). The primers used to amplify nt 509-674 of OCX-32 cDNA (Gautron et al., 2001) for sequencing exons 4-6 were 5'-cagagcacacgggttacttattgg (upper) and 5'- ctaatagggtgacctaatgt (lower). Accession numbers of the sequences compared in this study are: OCX-32 (AJ307060); RAR-TIG1: human (BC029640), mouse (XM_130987); latexin: human (BC008438), mouse (NM_016753), rat (NM_031655).

Results and discussion

Database searching with the protein sequence of OCX-32 (BlastP) revealed that it possesses limited identity to two apparently unrelated proteins, latexin and RAR-TIG1. Latexin is a CPA inhibitor which is expressed in some brain regions and in some non-neural tissues such as the peritoneal mast cell (Normant et al., 1995; Uratani et al. 2000). RAR-TIG1 is the putative translation product of tazarotene-induced gene 1, a retinoic acid receptor-responsive gene expressed in human skin (Nagpal et al., 1996). These human proteins have only a 30% identity to each other, so their evolutionary and functional relationship to OCX-32 and to each other is not clear. This issue was explored with multiple sequence alignment (ClustalW) to compare different mammalian sequences of latexin and RAR-TIG1 with each other and with OCX-32 (fig. 1). The latexin and RAR-TIG1 sequences form clearly distinct groups, with OCX-32 more similar to the RAR-TIG's. This impression was confirmed by phylogenetic analysis (fig. 2). Database searching of the chicken EST database (TblastN) to detect chicken sequences more similar to latexin or RAR-TIG1 were unsuccessful. Nothing is known about the properties of RAR-TIG1 protein, which was reported as a transcript whose expression levels in skin are regulated by tazarotene, an RAR □□-selective retinoid. The originally reported transcript (accession U27185, Nagpal et al., 1996) is a shortened version (exons 1-4 only, plus 4 amino acid residues derived from the adjacent intron) compared to the full-length transcript with exons 1-6 (accession BC029640) that aligns so completely with OCX-32 and mouse RAR-TIG1. The evolutionary relationship between these proteins was examined by identifying the exons coding for the aligned protein sequences to detect similarities in the structural organization of the mammalian genes. Human and mouse exon boundaries were identified by blastN searching of the public genome databases with the cDNA sequences to identify the intron/exon boundaries. Preliminary data from genomic sequencing of the chicken OCX-32 gene was also compared (fig. 3). The very close correspondence between all exon boundaries in latexins and RAR-TIG1s suggests that there is an evolutionary relationship between these two genes (ie. gene duplication followed by divergence), and with OCX-32. The human latexin/RAR-TIG1 genes are located within 60 kb of each other on chromosome 3.

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CPA inhibitory activity of latexin has been demonstrated for the rat recombinant protein; it was suggested that an 11-residue region possessing homology to the "activation segment" of potato CPA inhibitor is responsible for this activity (Normant et al., 1995). While all latexins are identical at these residues, much less conservation is seen for RAR-TIG1 and OCX-32 (fig. 4). Therefore, it remains an open question as to whether OCX-32 and RAR-TIG1 also have CPA inhibitory activity. Studies with purified eggshell or recombinant OCX-32 are necessary to determine its activity and function during eggshell formation.

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Figure 1. Protein sequence alignment between latexin, RAR-TIG1 and OCX-32. http://www.ebi.ac.uk/clustalw/

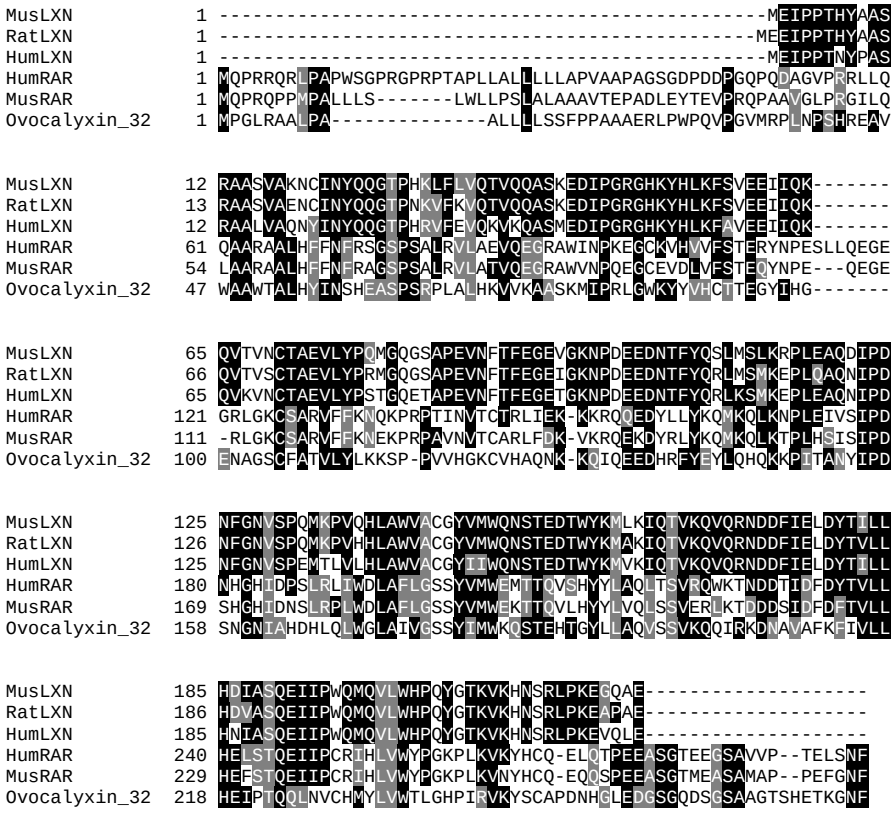

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Figure 2. Consensus tree for RAR-TIG1, latexin and OCX-32 sequences.

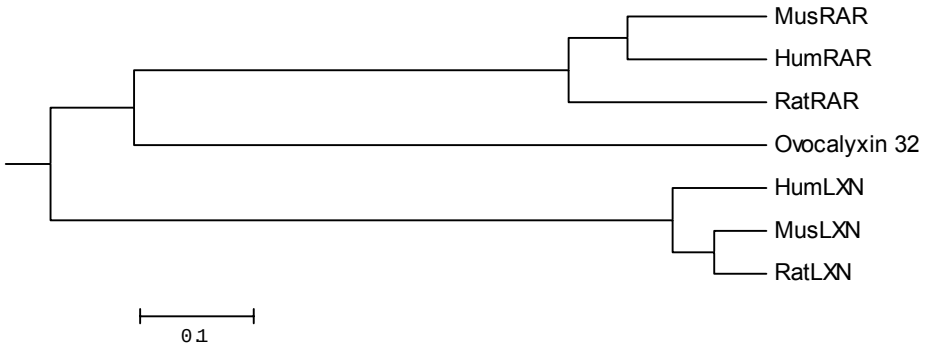

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Figure 3. Gene structure of human and rodent LXN and RAR, and preliminary results for OCX-32. Exons coding for aligned protein sequences are identified.

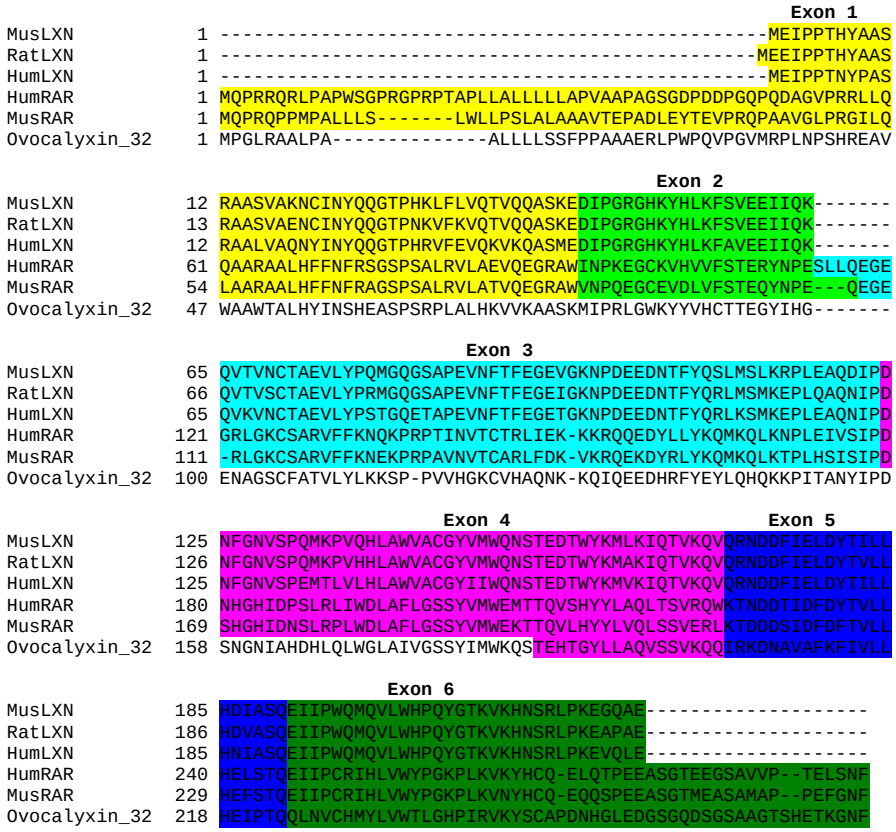
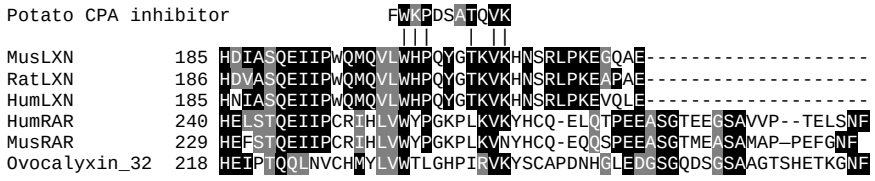


Figure 4. Comparison of potato CPA inhibitor "activator segment" with latexin, RAR-TIG1 and OCX-32.



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INFLUENCE OF MODEL PROTEINS ON THE PRECIPITATION OF CALCIUM CARBONATE

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Keywords : Crystals, Calcium carbonate, proteins, precipitation, polymorphs.

Abstract

Eggshell microstructure is suspected to be influenced by its organic matrix. To investigate the effect of proteins on calcium carbonate precipitation, we have used a group of globular proteins (lysozyme, ribonuclease, myoglobin, and α-lactalbumin) for a comparative study. At low concentrations, they favored the precipitation of calcium carbonate, increasing the number of crystals, whereas at higher concentrations they inhibited it, decreasing drastically the number of crystals and slowing nucleation. The four proteins studied favoured calcite precipitation over the other calcium carbonate polymorphs (aragonite, vaterite). The effect of all these proteins on the morphology of the crystals was the same, but some proteins (ribonuclease-A, α-lactalbumin) were more effective and produced the same effect at lower concentrations.

Résumé

La microstructure de la coquille serait influencé par sa matrice organique. Afin de cerner l'impact des protéines sur la précipitation du carbonate de calcium, nous avons utilisé un groupe de protéines globulaires (lysozyme, ribonucléase, myoglobine, and α-lactalbumin) afin d'effectuer une étude comparative. Pour de faibles concentrations, elles favorisent la précipitation du carbonate de calcium , augmentant le nombre de cristaux tandis que lorsque la concentration est élevée, elles l'inhibent, le nombre de cristaux diminue franchement et la nucléation se ralentit. Les quatre protéines étudiées favorisent la précipitation du calcite par rapport aux autres polymorphes de carbonates de calcium (aragonite, vatérite). L'effet de toutes ces protéines sur la morphologie des cristaux a été le même, mais certaines protéines (ribonucléase-A, α-lactalbumin) ont été plus efficaces et produisent le même effet à des concentrations plus faibles.

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Introduction

Organisms induce the precipitation of a great variety of biomineral tissues (e.g., eggshell, mollusk shell, bone, otoliths, etc). In the regulation of these structures, in particular in hens during the formation of eggshell, specific proteins are released at different times during their formation processes and play an important role in controlling the nucleation, polymorphic phase, morphology and orientation of individual crystals (Addadi & Weiner 1992; Nys et al 1999).
To investigate *in vitro* the effect of proteins on calcium carbonate precipitation, we have used a group of globular proteins (lysozyme [LZM], ribonuclease-A [RBN], myoglobin [MIO], and α-lactalbumin [α-LA]) for a comparative study. These proteins are of similar size and conformation, but have different isoelectric points, so that at a given pH their surface charge differs, and their adsorption behaviour on differents type of substrates is well characterized (Haynes and Norde, 1994). Differences in their adsorption behaviour are mostly caused by their different surface charges. The present study is a detailed examination of how these proteins affect the precipitation of calcium carbonate (i.e., nucleation, polymorphism and crystal morphology) and in particular how protein surface charge affects precipitation.

Material and Methods

The precipitation experiments were carried out at 20 ºC on isolated environmental chambers as described more in detail elsewhere (Jiménez-López et al. 2003). Calcium carbonate was precipitated in the absence of proteins (control experiments) and in the presence of proteins (protein-bearing experiments). Experiments were terminated at 48 hours, the chamber was opened and both solution and solid were withdrawn from the microbridges for chemical and morphological analyses.

Results and discussion

1. *Screening of the precipitation conditions*

First, a series of experiments was done to analyse the influence of system parameters on the precipitation of calcium carbonate. The two system parameters which varied were $CaCl_2$ and NH_4HCO_3 solution concentrations. The influence of these parameters on the number of crystals and relative abundances of polymorphs is illustrated in Fig. 1. The number of crystals increased with $CaCl_2$ and NH_4HCO_3 concentrations. Of

these two parameters, the concentration of NH_4HCO_3 had the greater influence on the number of crystals and relative abundance of polymorphs.
Also, system parameters affected the relative abundances of polymorphs, increasing the percentage of calcite with concentration of solutions.

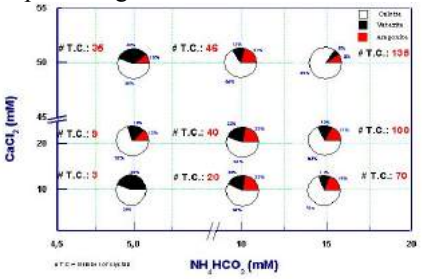

Fig. 1. Influence of $CaCl_2$ and NH_4HCO_3 solution concentrations on the number of crystals and relative abundances of polymorphs.

According to these results, we selected as working conditions for the subsequent protein-bearing experiments: 20 mM $CaCl_2$ and 10 mM NH_4HCO_3.

2. Effect of proteins on calcium carbonate precipitation

The presence of proteins affected the waiting time for precipitation, the number of crystals, the relative amount of the different polymorphs and the morphology of crystals. Generally, the presence of protein postponed the precipitation of calcium carbonate and at higher concentrations they suppressed totally the precipitation.

2.1 Number of crystals

The effect of the different proteins on crystal nucleation is illustrated in Fig. 2. LZM and α-LA (at low concentrations) favoured the nucleation of calcium carbonate crystals, increasing the number of crystals. RBN, MIO, and α-LA (at high concentrations) inhibited calcium carbonate precipitation, slowing nucleation and decreasing drastically the number of crystals. Among proteins, α-LA was the most effective at promoting a very intense nucleation even at very low concentrations of this protein (16-128 μg/mL).

2.2 Amount of precipitate

The total amount of calcium carbonate precipitated increased sharply with protein concentration reaching a maximum at around 128 μg/mL to decrease again sharply as protein concentrations increased further (Fig. 3). At higher protein concentrations (2000 μg/mL), the precipitation of was totally suppressed with the exception of LZM that did not attain total suppression until 50 mg/mL.

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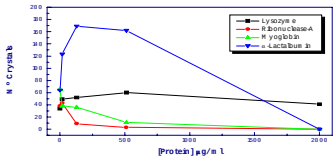

Fig. 2. Effect of the different proteins on crystal nucleation.

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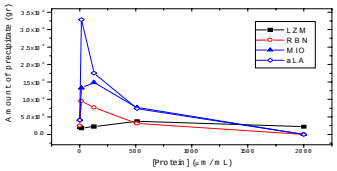
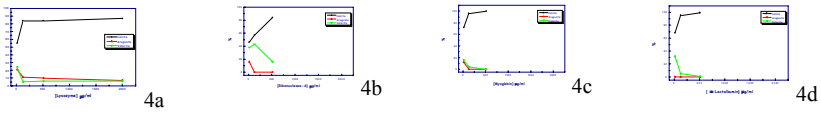


Fig. 3. Effect of different proteins on the total amount of calcium carbonate precitated.

The effect of the different proteins over precipitation varied. Precipitation rate increased following the sequence: LZM < RBN < MIO < α -LA.

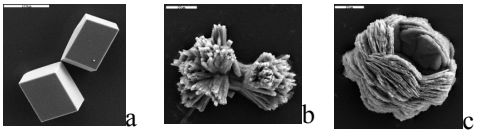
2.3 Polymorphic phase

All the proteins studied favoured calcite precipitation over the other polymorphs (Fig. 4). MIO and α-LA were the most effective in promoting calcite precipitation. Even at a low concentration of these proteins (128 μg/mL), calcite was the sole phase that precipitated.



4a 4b 4c 4d

Fig. 4. Effect of the different proteins on the polymorphic phase. Expriments were interrupted at 48 hours.



a b c

Figs. 5. Typical morphologies of crystals in the control experiment. a) calcite crystal rhombs, b) aragonite needle-like aggregate and c) vaterite leaf-like aggregate.

In general, the studied proteins induce the aggregation of crystals (particularly at high concentrations), and as protein concentration increases so does the size of aggregates. Proteins also affected crystal morphology. In the absence of protein (control samples), calcite crystals were always perfect rhombohedra. As the concentration of protein increased the morphology of calcite crystals was modified by the appearance of new crystal faces. The symmetry of the newly expressed faces corresponded to that of the {110}, {100}, {001} forms of calcite (Fig. 6 f and h). The effect of all these proteins on the morphology of calcite crystals was similar, but some proteins (RBN, MIO) were more effective and produced the same effect as the others did but at lower concentrations. Calcite crystals grown in the presence of α-LA were elongated along the ***a***-axis (Fig. 6h). As the protein concentration increased, they formed aggregates of

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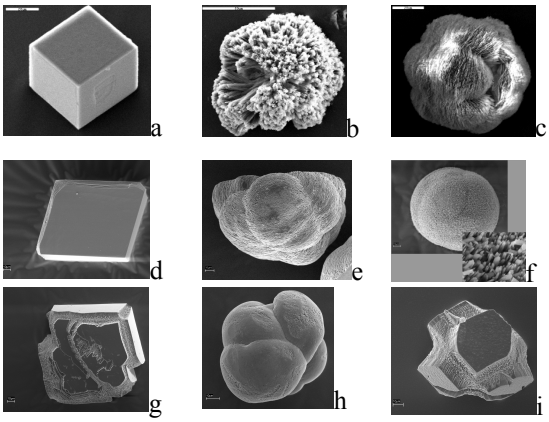
crystals. In the same conditions, RBN and MIO induced the formation of spherical aggregates (Figs. 6e, f and h). With respect to the other polymorphs (aragonite and vaterite), the most notable effect of this group of proteins on crystal morphology was a reduction in the size of crystals and a smoothing in the surface of the aggregates that they form.



Figs. 6 a, b and c; Calcite, aragonite and vaterite with 128 μg/mL lysozyme respectively.

Figs. 6. d, e; Calcite, and f Aragonite with 128 μg/mL ribonuclease.

Figs. 6 f, and g. Calcite, in presence of 128 μg/mL myoglobin and h Calcite, in presence of 128 μg/mL α-lactalbumin.

In summary, the present study is a detailed examination of how the studied proteins affected the precipitation of calcium carbonate (i.e., nucleation, polymorphism and crystal morphology). Proteins readily adsorb on to the incipient nuclei and crystal surfaces interfering with their growth.

Protein surface properties

Protein adsorption is affected by protein surface properties (electrical charge density, conformation and hydrophobicity) as well as solution conditions (pH, ionic strength, etc.). This is because these parameters affect protein structure. Adsorption is enhanced by hydrophobicity of the protein. Also, electrostatic interaction plays an important role, especially with hydrophilic surfaces such as calcite (Aradi and Norde, 1990). At the stabilization pH at the end of our experiments (pH 7.3), LZM, which has the highest isolectric point (pI 11) of all four proteins, is positively charged, RBN (pI 9) is also positively charged, MIO (pI 7) is not charged and α-LA (pI 4) is negatively charged. We observed that, depending on the nature of the charge on the protein, its morphological effect on calcium carbonate precipitation varied, with this effect becoming more pronounced when the protein is neutrally or negatively charged. This behaviour suggests that the dominant mechanisms in adsorption of this group of proteins are hydrophobic and electrostatic interactions, these proteins adsorbing to different faces in accordance with their net electrostatic attractions (or repulsions) to calcite surfaces. When proteins are neutral or low charged (like in the case of RBN

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and MIO) the dominant effect is hydrophobic interactions. Under this condition, the amount of protein adsorbed on the surface is a maximum, so that proteins became dehydrated by their adsorption on to a surface.

Effect of proteins on nucleation and precipitation rate

Depending on their concentration, proteins have opposite effects on calcium carbonate precipitation. At low concentration, proteins promoted calcium carbonate precipitation while at higher concentration they inhibited it. This ambivalent behaviour of proteins is characteristic of crystallization inhibitors that have a strong affinity for target crystal surfaces. At low concentrations of these substances, when there is an excess of material supply for crystal growth, they act as substrates promoting nucleation and favouring precipitation due to stereochemical affinity with the crystal surface (Lahav and Leserowitz, 1993). In contrast, at higher concentration, when there is an excess of inhibitors, they bind to the crystal surface growth sites and inhibit precipitation. Also, the effect of these inhibitor substances increases as they are more negatively charged. Alternatively, there are other possible mechanisms by which these proteins could affect calcium carbonate precipitation. First, proteins in solution locally raise Ca^{2+} concentration in those regions close to negatively charged patches on the protein surface (ionotropic effect; Addadi and Weiner, 1992). This creates local areas with higher saturation values than those of the bulk solution. Nucleation would be favoured in these areas, even when saturation in the bulk solution is not high enough to produce nucleation. Then, proteins could act as nucleation centers promoting nucleation and increasing the precipitation rate. The importance of the ionotropic effect should increase as the negative charge on the proteins increases, explaining the observed sequence of increasing precipitation rate with increasingly more negatively charged proteins. Second, some of these proteins (i.e., LZM, α-LA) that have an affinity for calcium (Creamer & MacGibbon 1996) could act as chelating agents, reducing the available calcium concentration in solution and the effective supersaturation. A reduced amount of Ca^{2+} exhausts crystal growth and inhibits precipitation of calcium carbonate. Third, the proteins could adsorb on to the incipient crystals and block their accessible surfaces, impeding further crystal growth. Finally, it seems that the ionotropic effect is more important at low protein concentrations where there is a more intense nucleation. This effect also increased with the negative charge of the proteins following the sequence LYZ, RIB, MIO, α-LA in which the precipitation of calcium carbonate increases. In contrast, at higher protein concentrations, the chelating effects of proteins and/or the adsorption of proteins to the growing surface were dominant, affecting negatively precipitation and suppressing it totally at about 2 mg/mL (with the exception of lysozyme).

Effect of proteins on polymorphism and crystal morphology

Proteins can also favour the precipitation of one polymorph over another. The group of proteins studied here had an effect on polymorphism. All four proteins favoured the precipitation of calcite over the other calcium carbonate polymorphs (aragonite,

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vaterite). Interestingly, α-LA is the most effective in selecting calcite and also the most negatively charged which made this protein a more powerful chelating agent.
Modifications in crystal growth (Addadi and Weiner, 1992) and dissolution behaviour can provide insights regarding protein-mineral surface interactions. For instance, in both cases modifications of the habit of calcite crystals are induced by the preferential adsorption of proteins to specific faces, which are expressed as a consequence of reductions in their growth (or dissolution) rate. Crystal growth experiments are a diversion from traditional protein adsorption experiments which use static substrates. In change, during crystal growth new surfaces are being created and proteins select those surfaces to which they have more affinity. Also, proteins preferentially interact with those crystal faces which have the maximum density of carbonate groups and in which the carbonate groups are oriented perpendicular to the surface. The sequential inhibition of the growth of the {110}, {100}, and {001} faces could be caused by the combined effect of the density of carbonate groups and their orientation (Jimenez Lopez et al. 2003).
In conclusion, we have observed that proteins close to being neutrally charged adsorb more strongly onto calcium carbonate crystals as deduced by their strong effect on morphology. In contrast, charged proteins, especially those negatively charged (α-LA) were more effective in controlling nucleation, having opposite effects depending on the concentration range. Also, negative proteins were more effective in selecting calcite over the other polymorphs of calcium carbonate.

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IDENTIFICATION OF 3 NEW MINOR HEN EGG WHITE PROTEINS

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Keywords : egg white, protein, Ex-FABP, CAL gamma, HEP21

Abstract

Because of the lack of analytical techniques for minor proteins identification, the composition of the egg white is not yet completed. The two-dimensional electrophoresis techniques and molecular biology techniques enable to isolate and characterise proteins present in very low concentrations. Using these techniques, we identified three minor proteins in hen egg white. The first one is Ex-FABP, also called Ch21 protein or quiescence-specific protein. The second one is CAL gamma, a lipocalin recently determined from nucleic acid sequencing. The third one was novel and called HEP21. No significant homology with already known proteins was observed, but HEP21 was identified as a new member of the uPAR/Ly6 protein superfamily ; the complete cDNA sequence and the tissue expression of this protein have been determined.

Résumé

La composition du blanc d'œuf n'est pas encore complètement déterminée, en raison notamment des limites des techniques d'analyse pour l'identification des protéines mineures. Mais l'électrophorèse bi-dimensionnelle et les techniques de biologie moléculaire permettent d'isoler et de caractériser les protéines présentes à très faible concentration. C'est ainsi que trois protéines mineures ont été identifiées dans le blanc d'œuf de poule. La première est la protéine Ex-FABP, également appelée protéine Ch21 ou quiescence-specific protein. La seconde est la protéine CAL gamma, dont la séquence a été récemment déterminée à partir de l'acide nucléique correspondant. La troisième, que nous avons dénommée HEP21, est une nouvelle protéine, sans homologie significative avec les protéines connues à ce jour, mais que nous avons pu

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rattacher à la super-famille protéique uPAR/Ly6 ; la séquence complète de l'ADNc et l'expression tissulaire de cette protéine ont été déterminées.

Introduction

The biological role of the egg white proteins is well documented, but essentially on components which are quantitatively predominant, and involved in a limited number of activities. And it is increasingly obvious that egg white very likely contains a great number of molecules involved in the complex regulatory phenomena that takes place during embryo development (Stevens, 1996). Thus, egg white could be seen as a very rich medium, with numerous and various biological activities, possibly of interest to the medical and pharmaceutical sectors, as well as for the understanding of the biological phenomena which could lead to the preparation of bioactive fractions.
However, this research is now confronted with limited knowledge about the precise composition of egg white. Only about 15 proteins have been identified in this apparently ordinary biological liquid (Li-Chan and Nakai, 1989).
With the recent developments of new analytical methods for the characterization of proteins (two-dimensional electrophoresis techniques, molecular biology techniques and mass spectrometry), it is now possible to isolate, and to characterize components present in very low concentrations. A large number of minor proteins, not yet identified, have been revealed in hen egg white (Désert et al., 2001). The study reported here the isolation and the identification of three minor acidic egg white proteins.

Material and methods

Hen egg white proteins were subjected to high-resolution two-dimensional electrophoresis on a pH 4 to 7 linear gradient, with a second-dimension 12% acrylamide gels and detection with Coomassie blue R250, as already described (Désert et al., 2001).
The protein spots were excised out from several identical semi-preparative 2-D gels, pooled and sent to Institut Pasteur (Laboratoire de Microséquençage des Protéines, Paris, France) for sequence analysis after tryptic digestion, as already described (Nau et al., 2003)
The proteins separated by 2D-PAGE were blotted on Immobilon P^{SQ} membrane in a semi-dry Trans-Blott Cell system. After staining, the considered spots were cut out from the membrane for N-terminal sequencing by automated Edman degradation.

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The RNA samples were extracted from the magnum (RNA InstaPure kit) and were reverse transcripted by standard techniques (Sambrook and Russel, 2001). The primers used for the amplification experiments were deduced from peptides sequences or cDNA sequences previously determined, as indicated in the results.
5' RACE-PCR were performed using the SMART^{TM} RACE cDNA Amplification Kit (Clontech). The gene-specific anti-sense primers were deduced from the encoding fragments previously obtained and sequenced, and used in association with the UPM primer provided.
The PCR amplified cDNA products were analyzed on 1% agarose gels stained with ethidium bromide, and sequenced.
For tissue expression of HEP21, total RNA was extracted from the different parts of the oviduct (magnum, white and red isthmus, shell gland) and from various tissues (liver, kidney, duodenum, brain, spleen and bursa of Fabricius) for northern blotting by standard techniques. A 400 bp PCR product was amplified using specific HEP21 primers and was then radiolabeled (^{32}P) to be used as a cDNA probe.

Results and discussion

2D-electrophoresis allowed the detection of many minor proteins (Désert et al., 2001). Among the higher intensity unidentified proteins, we paid particular attention on 3 spots, localized in the gel area corresponding to small (MW < 25 kDa) and slightly acidic proteins (pI about 6) (Figure 1).

Identification of two chondrogenesis-related lipocalins

Sequencing of tryptic peptides digests from spot 1 and 2 revealed ISFLGED and ETNYDEYALVATQ amino acid sequences, respectively. They corresponded to the fragment 60-66 of extracellular fatty acid binding protein (Ex-FABP), also called Ch21 protein or quiescence-specific protein (accession number P21760), and to the fragment 117-129 of CAL gamma (accession number Q8QFM7), respectively. Both of them are polypeptides of the lipocalin protein family, which genes are located within the same genomic cluster (Pagano et al., 2003).
Ex-FABP was initially found in chick embryo skeletal tissues and is suspected to be a stress protein expressed during chondrocyte and myoblast differenciation (Descalzi-Cancedda et al., 2001). Its relation with cartilage formation and inflammatory responses has been demonstrated (Descalzi-Cancedda et al., 2000). But it was also recently observed for the first time in hen egg white through SDS-PAGE analysis (Larsen et al., 1999). The apparent molecular weight determined in the present study (23 kDa) was slightly higher than that estimated by these authors and significantly higher than its theoretical molecular weight (18 kDa) deduced from the genomic sequence. However, its apparent pI (5.6) is consistent with its theoretical one,

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calculated from the amino acid sequence (pI 5.2). The determined N-terminal amino acid sequence (TVPDRS) of the digest was different from the both identified for the Ch21 secreted in chondrocytes (AATVPDRS and ATVPDRS) (Descalzi-Cancedda et al., 1990). This result suggests that the proteolytic cleavage could be different in the egg white protein.

On the other hand, only the gene of CAL gamma protein has been identified and characterised (Pagano et al., 2003). Because of their analogous and peculiar developmental patterns of expression in chicken embryos, these authors suggested a synergistic action of CAL gamma and Ex-FABP in the process of endochondral bone formation. The present study indicated that this protein also exists in unfertilised hen egg white. As for Ex-FABP, the determined apparent molecular weight (23 kDa) was significantly higher than its theoretical one (20.8 kDa), but its apparent pI (6.1) is consistent with its theoretical one (6.3). It is thus more than likely that Ex-FABP and CAL gamma undergo posttranslational glycosylations responsible for their molecular weight upshifts.

Cloning and characterisation of HEP21

The spot 3 tryptic digest revealed unknown peptide sequences. One of them (YKIPYV) was used to design one degenerate primer for cDNA amplification, using an anchored polydT. The resulting 450 bp fragment was sequenced. One of the three putative open reading frames enabled matching of the VTLY peptide initially sequenced. The stop codon could then be localised, and thus one 189 nucleotides coding sequence identified, which corresponded to the 63 C-terminal amino acids. The missing 5' fragment was amplified using 5'RACE-PCR with UPM primer and a specific reverse primer deduced from the 3' end of the encoding sequence. The sequence of the resulting product is perfectly overlapping the previously determined fragment. The start codon was localised and consequently the complete nucleotide (318) and amino acid (106) sequences determined. Moreover, the N-terminal sequence (LQCKVCKY) indicated that the fragment 1 to 18 could correspond to a signal peptide. Searching for cDNA and protein homologies using the NCBI and EMBL databanks revealed that this sequence represents a novel protein that we called HEP21 (figure 2). Search of domain signature patterns clearly highlighted that HEP21 is a new member of the multifunctional uPAR/CD59/Ly-6/snake toxin family. This family is characterised by a common cysteine-rich domain of 70 to 90 amino acids with 8 to 10 regularly spaced cysteines capable of forming 4 to 5 disulfide bonds and conferring a specific three-dimensional structure to these proteins (Ploug et al., 1993). Moreover, the uPAR/Ly-6 domain contains a highly conserved short consensus sequence (CCxxDxCN) that also exists in HEP21. Nevertheless, unlike most of the known members of the uPAR/Ly-6 superfamily, HEP21 would be secreted, and not GPI-anchored, and predominantly expressed in a specific tissue, which is the magnum (Figure 3).

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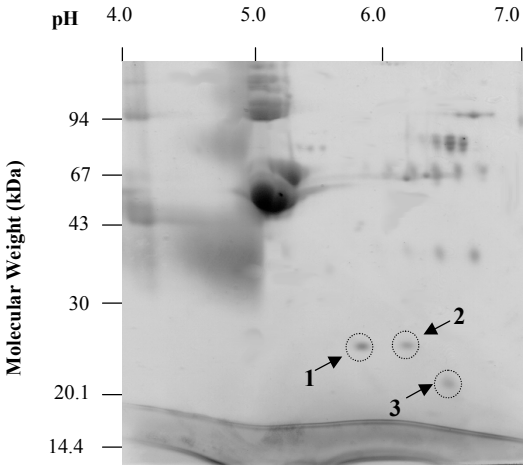

Figure 1- 2D-PAGE analysis of 1,000 µg of hen egg white proteins. Spot 1 : ex-FABP, spot 2 : CAL gamma protein, spot 3 : HEP21.

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-1  M   K   L   L   F   V   G   L   A   L   V   L   C   V   G   V   V   E   A ↓ L
 -3 ATG AAG CTG CTC TTT GTT GGG CTG GCC CTT GTC CTG TGC GTT GGA GTG GTT GAA GCT CTT

 20  Q   C   K   V   C   K   Y   K   I   P   Y   V   G   C   F   H   G   A   N   E
 58 CAG TGC AAG GTC TGC AAA TAC AAA ATC CCC TAC GTT GGG TGC TTC CAT GGG GCC AAC GAG

 40  T   T   C   E   R   R   E   R   C   A   I   I   K   T   S   L   G   K   V   T
118 ACC ACC TGC GAG CGG AGG GAG AGA TGT GCC ATC ATC AAA ACC TCC CTC GGG AAG GTG ACC

 60  L   Y   Y   Q   Q   G   C   T   S   A   L   N   C   G   R   E   R   A   S   D
178 CTC TAT TAC CAA CAA GGC TGC ACC TCT GCC CTG AAC TGT GGC AGG GAG AGG GCA TCA GAC

 80  A   E   S   R   L   T   S   R   Y   S   C   C   E   T   D   L   C   N   E   K
238 GCG GAA TCC CGT TTG ACA TCC CGG TAT TCC TGC TGC GAG ACC GAC CTG TGC AAC GAG AAG

100  W   D   D   D   P   T   D   *
298 TGG GAT GAT GAC CCC ACA GAT TAA
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Figure 2- Nucleotide sequence of cDNA and deduced amino acid sequence of HEP21. The arrow and the asterisk indicate the signal peptide cleavage site and the stop codon, respectively.

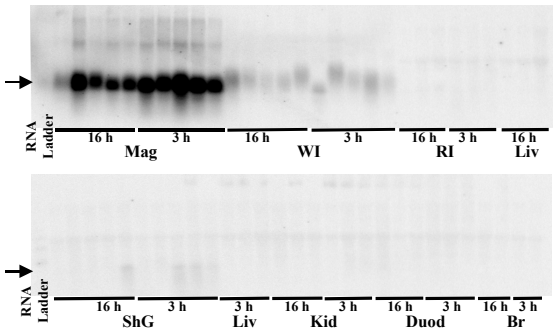

Figure 3- Northern blotting for expression of HEP21. Total RNA were prepared from magnum (Mag), white isthmus (WI), red isthmus (RI), shell gland (ShG), liver (Liv), kidney (Kid), duodenum (Duod) and brain (Br). Each lane represents RNA samples prepared from different hens. Tissues were collected 3h and 16 h post-oviposition.

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AMINOACIDS COMPOSITION AND PROTEIN PROFILE IN FREEZE-DRIED CHICKEN EMBRYO EGGS WITH DIFFERENT PERIODS OF DEVELOPMENT

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Keywords : embryo eggs, aminoacids composition, protein profile

Abstract

This article presents aminoacids composition and protein profile in freeze-dried chicken embryo eggs with different development periods (0, 3, 5, 7, 9, 11 days). The protein profile was determined through reverse phase-HPLC- and SDS-PAGE. Differences found in aminoacids composition were not significant, except for aminoacid proline. Protein profile of these eggs changes according to the period of development and the change is more evident after the 5th day, where there was an increase in ovalbumin, ovotransferrin, apoLDL, apoHDL and lysozyme concentration, and a decrease in ovomucin and ovostatin concentration. The hypothesis that the change in the protein profile is due to protein biosynthesis and disintegration that happens during incubation process is discussed.

Résumé

Ce travail présente la composition en acides aminés et le profil protéique des oeufs embryonnés de poulet lyophilisés à différents moments de la couvaison (0, 3, 5, 7, 9, 11 jours). Le profil protéique a été évalué par HPLC en phase inverse et par SDS-PAGE. Nos résultats montrent qu'il n'y a pas de différences dans la composition en acides aminés à l'exception de la proline. Le profil protéique de ces oeufs n'est pas identique et la différence est plus évidente à partir du 5ème jour de développement, où il y a une augmentation de la concentration en ovalbumine, ovotransferrine, apoLDL, apoHDL, lysozyme et une diminution de la concentration en ovomucine et ovostatine.

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L'hypothèse que le changement du profil protéique est dû à la biosynthèse et à la dégradation des protéines lors de la couvaison est discutée.

Introduction

Since ancient times chicken embryo eggs, fertile eggs and unfertilized eggs have been used by humanity as food and also in the treatment of diseases. From the nutrition point of view, eggs were always one of the most complete foods available for man (Thapon and Bourgeois, 1994).
Many researchers have shown that eggs, besides being a source of several nutrients, can provide active substances for therapeutic and diagnostic uses (Stadelman, 1999).
The chicken embryo eggs have been used by Brazilian Amazon area inhabitants as a treatment for serious bacterial origin pathologies such as tuberculosis, but this has not been scientifically proven yet. Trying to prove scientifically these popular and secular uses, we intend to search some active substances and essential nutritional elements in chicken embryo eggs, in order to contribute to the study of potential uses in the development of foods and/or medicines.
This work was designed to evaluate the aminoacids composition and to characterize proteins in freeze-dried chicken embryo eggs with different periods of development to know if there are some differences between them.

Material and Methods

Preparation of sample units: Chicken embryo eggs (*Gallus gallus domesticus* L.) were obtained from *Isabrown* fertile eggs, incubated at $39^{o}C \pm 0.5^{o}C$ for 0, 3, 5, 7, 9 and 11 days. The *Isabrown* hens were bred in intensive outdoor raise system, according to Salatin (1993). The shell of incubated eggs was removed aseptically in sterile plates and the stage of development of the embryo was verified. The remaining content was homogenized, frozen and freeze-dried. The freeze-dried product was packed, sealed and labeled. Samples of the flasks were selected for analysis using random numbers (Vieira and Hoffmann, 1989).
Aminoacids composition : Total aminoacids composition of each sample was determined according to Spackman *et al*. (1958).

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Protein profile by reverse phase-HPLC : Protein characterization in freeze-dried chicken embryo eggs with different periods of development was determined by reverse phase-HPLCaccording to Nau *et al*. (1999).

Protein profile by SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (1970), using a 15% acrylamide separating gel containing 0.1% of SDS. The gel was stained for proteins by using the Coomassie blue method (Tunon and Johansson, 1984).

Results and discussion

There are not significant differences in aminoacids composition except for proline (Table 1).

Table 1: Average of aminoacids composition (g/Kg) in sample units of freeze-dried chicken embryo eggs (*Gallus gallus domesticus* L.) with different incubation periods.

AA/days	0	3	5	7	9	11
Asp	46,0	51,2	43,0	49,6	53,2	51,9
Thr	21,7	24,6	22,2	26,2	24,7	22,4
Ser	32,5	34,8	31,2	34,2	36,6	33,5
Glu	59,3	74,0	55,1	60,5	62,5	61,4
Pro	12,6	9,4	8,0	9,9	15,8	19,1
Gly	16,8	18,5	15,1	18,7	15,1	14,5
Ala	26,0	29,2	23,5	24,9	23,1	24,6
Cys	10,9	11,9	11,3	14,9	12,8	13,8
Val	26,8	30,8	29,0	31,4	24,5	25,3
Met	15,0	16,7	19,6	18,3	14,8	16,0
Ile	21,1	23,8	19,0	24,4	21,2	19,5
Leu	36,7	31,6	35,6	42,4	30,8	30,7
Tyr	19,3	25,2	20,0	26,1	21,7	23,2
Phe	31,0	35,9	29,7	36,7	27,1	25,3
His	11,1	11,8	9,0	9,5	11,2	11,4
Lys	28,0	31,7	25,2	27,1	30,4	32,1
NH3	7,0	7,2	5,8	6,0	6,6	6,3
Arg	28,4	30,9	30,4	28,7	31,6	29,6

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We also verify that embryo eggs with different development periods can be a source of essential aminoacids such as threonine, valine, methionine, isoleucine, phenylalanine, histidine, and arginine.

Protein concentration of embryo eggs changes according to development time, and this variation is more important from the 5th day because of protein biosynthesis and disintegration that happens during incubation process (Figure 1).

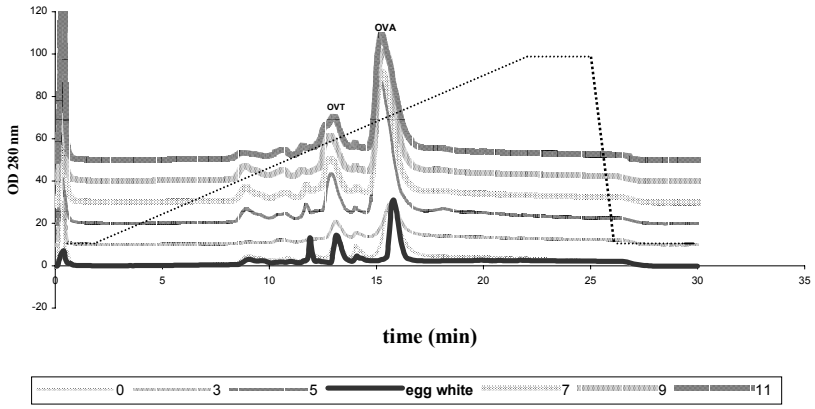

Figure 1: RP-HPLC chromatogram of freeze-dried chicken embryo eggs proteins with different incubation days.Conditions: sample units of embryo eggs were re-hydrated in H_2O Milli-Q, liquid egg white as reference; column: Vydac C4; elution with water-acetonitrile 0.025% TFA (dashed lines indicate the concentration of acetonitrile in the phase); flow rate: 0.8 ml/min; detection: 280 nm. Due to the graphic line being put upon, 10 points were added in OD (ordinates axis), for every incubation day (starting from the 3rd day), for a better visualization of the chromatogram.

The two highest peaks, identified as OVT and OVA, correspond to ovotransferrin and ovalbumin, respectively, because their retention times are coincident with those obtained in liquid egg white. The peak marked OVA, corresponds to that found by Croguennec *et al.* (2000), where pure ovalbumin was eluted with a retention time of about 15 minutes by RP-HPLC.

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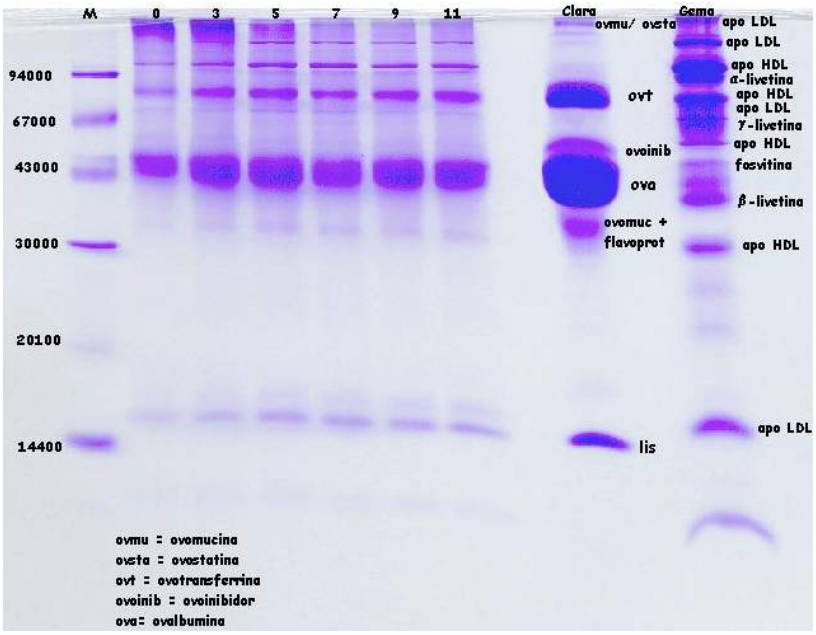

Figure in english, please !!!!!

Figure 2: Characterization of proteins in freeze-dried chicken embryo eggs with different incubation days by SDS-PAGE.Coomasie blue staining method. Deposit: 40 µl at 2.5 µg/µl. M= markers of molecular weight.
0, 3, 5, 7, 9 and 11 = embryo eggs with different development periods.
Egg white and yolk as references.

Bands shown in Figure 2 correspond to protein fractions: ovomucin, ovostatin, apoLDL, apoHDL, α-livetin, ovotransferrin, apoLDL, ovoinhibitor, ovalbumin, phosvitin, β-livetin, ovomucoid + flavoprotein, apoHDL and lysozyme, respectively. Molecular weights of these bands are coincident with those published by Stevens (1991) and with data of Awandé *et al.* (1994) and Croguennec *et al.* (2000).

Figure 2 also indicates that there are differences in protein characterization in freeze-dried chicken embryo eggs with different incubation days, and the difference was more evident following the 5th day of development, where there was an increase in ovalbumin, ovotransferrin, apoLDL, apoHDL, lysozyme concentrations and there was a decrease in ovomucin and ovostatin concentrations.

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CONTENTS

COMPOSITION OF ω-3 AND ω-6 FATTY ACIDS IN FREEZE-DRIED CHICKEN EMBRYO EGGS WITH DIFFERENT DAYS OF DEVELOPMENT

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Keywords : chicken embryo eggs, fatty acids ω-3 and ω-6, DHA.

Abstract

Fatty acids ω-3 and ω-6 composition and specially DHA were determined in freeze-dried chicken embryo eggs with pre-determined incubation periods. There were no significant differences in total contents of ω-3 fatty acids (p=0.1226) between freeze-dried chicken embryo eggs with different incubation periods (3, 5, 7, 9, and 11 days) and fertile freeze-dried chicken eggs (day 0). However, there were significant differences in total averagecontents of ω-6 fatty acids (p=0.0001). There was also a strong statistical evidence that values of DHA content fitted a quadratic model (p= 0.0013).

Résumé

Dans ce travail ont été determinées les compositions en acides gras ω - 3, ω - 6 et, particulièrement, en DHA, dans des oeufs embryonnés de poulet lyophilisés après différentes périodes de couvaison. Nous avons observé qu'il n'y a pas de différences considérables dans les teneurs moyennes totales en acides gras ω-3 (p=0,1226), entre les oeufs embryonnés de poulet lyophilisés après différentes périodes de couvaison (3, 5, 7, 9 et 11 jours) et les oeufs fertiles lyophilisés (jour 0). Cependant, il y a des différences considérables dans les teneurs moyennes totales en acides gras ω-6 (p=0,0001). Les valeurs de DHA obtenues après les différentes périodes de couvaison suivent un modèle de régression non linéaire quadratique (p=0,0013).

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Introduction

Since ancient times chicken embryo eggs, fertile eggs and unfertilized eggs have been used by humans as food and also in the treatment of diseases. From the nutrition point of view, eggs have been always one of the most complete foods available for man (Thapon and Bourgeois, 1994). Besides vitamins and mineral elements, eggs can provide three essential elements for a good diet: proteins, lipids and carbohydrates (Beig and Garcia, 1986).

In recent times, cardiovascular diseases in occidental populations have increased and, consequently, caused an increase in people's interest for foods that have the capacity of preventing these pathologies. Polyunsaturated fatty acids, mainly those of ω-3 family, such as docosahexaenoic acid(DHA), have a very important role in these diseases prevention, because of their property of decreasing the blood pressure and being reducers of rates of blood plasma's triglycerides. Other effects attributed to ω-3 fatty acids included an increase of memory capacity, visual development, and formation of cerebral tissue (Park *et al.*, 1997). ω-3 fatty acids, therefore, are considered benefic and researches have been trying to increase their quantity in food, specially eicosapentaenoic acid (EPA), or docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) (Stadelman and Pratt, 1989).

Ferrier *et al*. (1995) mentioned that eating eggs enriched with ω-3 fatty acids produced significant increase in docosahexaenoic acid (DHA) concentration and other polyunsaturated ω-3 fatty acids in phospholipid fraction of human blood.

Embryo chicken eggs could be a natural source of ω-3 fatty acids, mainly of DHA, because several studies have demonstrated that embryo's fatty acids and lipids composition is completely different from original yolk, from which embryo is derived (Speake *et al*., 1998).

Material and Methods

Preparation of sample units: Chicken embryo eggs (*Gallus gallus domesticus* L.) were obtained from *Isabrown* fertile eggs, incubated at $39^{o}C \pm 0.5^{o}C$ for 0, 3, 5, 7, 9 and 11 days. The *Isabrown* hens were bred in intensive outdoor raise system, according to Salatin (1993). The shell of incubated eggs was removed aseptically in sterile plates and the stage of development of the embryo was verified. The remaining content was homogenized, frozen and freeze-dried. The freeze-dried product was packed, sealed and labeled. Samples of the flasks were selected for analysis using random numbers (Vieira and Hoffmann, 1989).

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Fatty acids composition: Fatty acids were determined by gas chromatography (AOCS, 1996) adapted by Abril and Barclay (1999), in which fatty acids are identified by comparison of pattern retention times and quantified by areas standardization.
Statistical design: For analyzing the data, variance analysis with contrasts (??) and regression analysis was applied using software Statistica for Windows version 5.1 and SAS System for Windows version 6.12.

Results and discussion

Table 1 showed that fertile and embryo eggs could be sources of saturated fatty acids: palmitic (average: 23.18 ± 0.54%) and stearic (average: 7.70 ± 0.28%); unsaturated: palmitoleic (average: 3.00 ± 0.19%), oleic (average: 36.28 ± 0.58%), linoleic (average: 22.18 ± 0.34%), linolenic (average: 1.08 ± 0.04%), arachidonic (average: 2.04 ± 0.03%) and docosahexaenoic (average: 0.91 ± 0.03%). These results were similar with average values found by Ferrier *et al.* (1995) for fatty acids composition in eggs used as control.

Table 1: Average of fatty acids composition in sample units of freeze-dried chicken embryo eggs *(Gallus gallus domesticus* L.) with different incubation periods.

Fatty acids (%)		0	3	5	7	9	11
C14:0	Miristic	0,32	0,30	0,36	0,32	0,32	0,27
C16:0	Palmitic	23,91	23,11	23,11	23,27	22,27	23,43
C16:1	Palmitoleic	3,00	3,02	3,19	3,00	3,14	2,64
C18:0	Stearic	7,66	7,87	7,37	7,77	7,43	8,12
C18:1	Oleic (ω - 9)	35,78	36,59	35,68	36,13	37,25	36,26
C18:2	Linoleic (ω - 6)	21,90	22,14	22,71	22,13	22,39	21,78
C18:3	Linolenic (ω - 3)	1,11	1,01	1,09	1,10	1,11	1,05
C20:3	Eicosatrienoic (ω - 6)	0,45	0,40	0,41	0,38	0,37	0,40
C22:0	Behenic	0,26	0,25	0,27	0,28	0,27	0,26
C20:4	Arachidonic (ω - 6)	2,02	2,09	2,04	2,05	2,00	2,05
C22:5	Docosapentaenoic (ω - 3)	0,22	0,16	0,37	0,30	0,30	0,30
C22:6	DHA (ω - 3)	0,95	0,89	0,86	0,90	0,91	0,94
Total of acids ω-3		2,28	2,06	2,32	2,30	2,32	2,29
Total of acids ω-6		24,37	24,63	25,16	24,56	24,76	24,23

(for the table, points instead of comma)

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Operating conditions: gas chromatograph - Perkin Elmer 8420; capillary silica column - CP-Sil-88, Nº 985132, 50 m x 0,25 mm; oven temperature: 160°C–10 min, 160-200°C (4°C/min), 200°C–20 min; detector temperature: 300°C; injector temperature: 270 °C; 1.0 μL injection; helium carrier gas (He): 45 psi – gas flow 0.98mL/min; Split: 1:55.

Figure 1 shows the contents of ω-3 and ω-6 fatty acids according to incubation periods in freeze-dried chicken embryo eggs. Results indicated that fertile and embryo eggs could represent a source of polyunsaturated fatty acids, including ω-3 fatty acids.

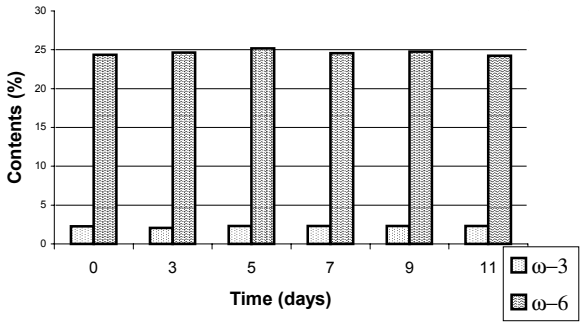

Figure 1: Content of ω-3 and ω-6 fatty acids versus development period in freeze-dried chicken embryo eggs.

Table 2 shows the variance analysis with the effect of the incubation period (0 day versus 3, 5, 7, 9 and 11 days) on the total average contents of fatty acids ω-3 and ω-6 in freeze-dried

Table 2: Variance analysis with effect of the incubation period (0 day versus 3, 5, 7, 9 and 11 days) on the total average contents of fatty acids ω-3 and ω-6 in freeze-dried eggs

It is not the good table: inversion between table 2 and table 3 !!!

source of variation	F	p-value
linear	0,13	0,7347
quadratic	31,99	0,0013*

There are no significant differences in total average contents of ω-3 fatty acids (p=0.1226), between freeze-dried chicken embryo eggs with different incubation periods (3, 5, 7, 9, and 11 days) and fertile freeze-dried chicken eggs (day 0) (Table 2). These embryo eggs presented an average content of these acids of 0.022% less than fertile eggs. However, there were significant differences in total average contents of ω-

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6 fatty acids (p=0.0001), between freeze-dried chicken embryo eggs with different incubation periods and freeze-dried fertile eggs. Embryo eggs presented an average content of these acids of 0.294% more than fertile eggs.
Noble and Cocchi (1990) found that: there were changes in composition of ω-6 and ω-9 fatty acids during incubation process, the composition and distribution of fatty acids from triglycerides fraction in yolk and yolk sac membrane remained unaltered and, there were expressive changes in cholesteryl ester fraction and phospholipids. Researches have shown that more than 90% of total energy required by embryo is supplied by β-oxidation of fatty acids derived from yolk lipids (Noble and Cocchi, 1990; Cherian and Sim, 1993 and Speake *et al.*, 1998).

Table 3: DHA content regression analysis in freeze-dried chicken embryo eggs with different incubation periods.
Table 2 and table 3 are inverted !!!

Contrast (?)	estimate	F	p-value
ω-3	- 0,022	3,23	0,1226
ω-6	0,294	617,40	0,0001*

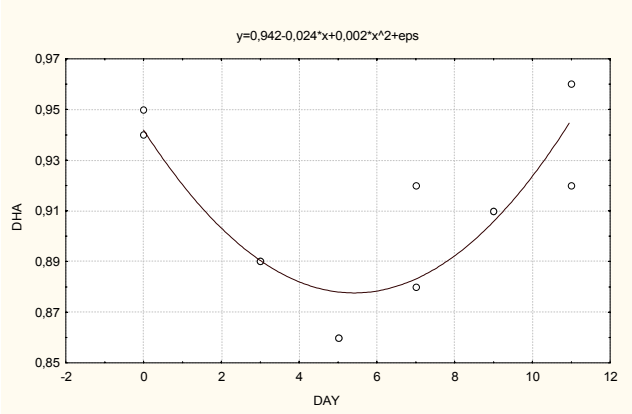

Figure 2: Scatter diagram of DHA average content (%) in sample units of freeze-dried chicken embryo eggs with different incubation periods.

Table 3 and Figure 2 represented regression analysis and scatter diagram of DHA content in freeze-dried chicken embryo eggs with different incubation periods. There

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was strong statistical evidence that quadratic model was related to expected values of DHA content (p=0.0006) and it originated the following equation:
% DHA = 0,942 – 0,02 days + 0,0022 days^2
Results allowed to deduce that chicken embryo development among 0 and 11 days of incubation did not change the average total contents of ω-3 acids, but changed the average total contents of ω-6 acids. Starting from the 5th day of incubation, DHA was preferably removed from the yolk before the largest period of the embryo's growth, for being associated to the growth and the development of the tissues and organs of the nervous system. And so, fertile and embryo eggs could be useful in human diet as sources of long chain ω-3 (DHA) and ω-6 (arachidonic acid) fatty acids.

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CONTENTS

ANALYSES OF EGG WHITE PROTEINS FROM DIFFERENT SPECIES BY CHROMATOGRAPHIC AND ELECTROPHORETIC METHODS

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Keywords : egg white protein analysis, RP-HPLC, SDS-PAGE, capillary electrophoresis

Abstract

This work describes the use of different high-resolution techniques for the study of egg white proteins from hen, quail, duck, pheasant and ostrich. For this purpose, RP-HPLC, SDS-PAGE, SDS-capillary gel electrophoresis (SDS-CGE) and free zone capillary electrophoresis using an uncoated capillary at pH 10.0 (CZE) were compared for egg white protein analysis. The major proteins: lysozyme, ovalbumin, ovomucoid and ovotransferrin were separated and identified using the four techniques, although differences were noted regarding the resolution provided by the different systems. Qualitative and quantitative differences were observed among the individual proteins of the animal species investigated.

Resumé

Cet article présente différentes techniques d'analyse hautement résolutives pour l'étude des protéines du blanc d'oeuf de poule, de caille, de canard, de faisan et d'autruche. La chromatographie liquide en phase inverse (RP-HPLC), l'électrophorèse en milieu SDS (SDS-PAGE), l'électrophorèse capillaire sur gel de SDS (SDS-CGE) et l'électrophorèse capillaire de zone (CZE) à pH 10 ont ainsi été comparées. Les protéines majeures : lysozyme, ovalbumine, ovomucoide et ovotransferrine ont été séparées et identifiées avec les quatre techniques, bien que des différences aient été observées entre les systèmes en terme de résolution. . Nous avons également observé des différences qualitatives et quantitatives entre les protéines individuelles des espèces étudiées.

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Introduction

Chicken eggs constitute one of the major protein sources of the diet (Powrie and Nakai, 1986). Egg white (albumen) accounts for about 58% of the entire egg mass and contains 10-12% proteins, mainly ovalbumin, ovomucoid, globulins, conalbumin and lysozyme. In addition to their nutritional importance, egg white proteins possess multiple functional properties such as foaming, emulsification and heat setting. This makes egg white an essential ingredient for the food industry (Mine *et al.*, 1995). Furthermore, new uses of egg-white proteins are increasingly being found in the production of health-promoting products, which contribute to enhance their traditional food value. Nevertheless, and despite its importance, hen egg, and particularly egg white, is surprisingly uncharacterized (Desert *et al.*, 2001), and even less is known on egg proteins from other poultry species.

Several chromatographic and electrophoretic techniques have been applied to the study of protein components of egg white (Takeuchi *et al.,* 1992; Chen and Tusak, 1994; Desert *et al.*, 2001; Recio *et al.,* 2001). This work compares different high-resolution techniques, such as RP-HPLC, SDS-PAGE, SDS-capillary gel electrophoresis (SDS-CGE) and capillary zone electrophoresis (CZE) for the analysis of egg white proteins from different poultry species.

Materials and Methods

Samples

Egg white samples were obtained in the laboratory from fresh shell eggs of different poultry species (hen, pheasant, quail, duck and ostrich), lyophilized and stored frozen until used. Their nitrogen contents were determined by the Kjeldahl method and protein contents were calculated by using a conversion factor of 6.25.

Egg white protein standards were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Molecular weight markers were from Pharmacia (Uppsala, Sweden).

RP-HPLC

RP-HPLC separations were carried out on a Beckman System Gold HPLC equipment with a System Gold Software data acquisition program 7.11 (Beckman Instruments, Fullerton, CA, USA), with a Hi Pore C_{18} column (250 x 4.6 mm, BioRad Laboratories, Hercules, CA, USA). Solvent A was 0.1% (v/v) TFA in HPLC grade water and solvent B was 0.1 % (v/v) TFA in HPLC grade acetonitrile. Elution was at flow rate of 0.8 ml min^{-1}, at room temperature, with a linear gradient from 2% to 65% of solvent B in 60 min. Absorbance was monitored at 214 nm

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SDS-PAGE

SDS-PAGE was performed using the PhastSystem Electrophoresis apparatus, Precast Phastgels Homogeneus 20%, and Phastgel SDS buffer strips (Pharmacia, Uppsala, Sweden). Electrophoretic and staining conditions with Phastgel Blue R followed the procedures of the manufacturer. Samples were dissolved in 10 mM Tris-HCl buffer, pH 8.0, containing 25 g L^{-1} SDS, 10mM EDTA, and 50 ml L^{-1} β-mercaptoethanol, and heated at 95 ºC for 10 min.

Capillary Zone Electrophoresis

Capillary zone electrophoresis (CZE) was performed with a Beckman P/ACE System MDQ, following the method described by Chen and Tusak (1994), with some modifications. Separations were carried out at pH 10, using an uncoated fused-silica capillary (NFL-06A, Composite Metal Services, LTD) of 0.40 m x 50 µm i.d. (0.30 m to detection point), with a slit opening of 100 x 800 µm. Migrations were run at 25ºC, with a linear voltage gradient of 0-10 kV in 3 min, followed by a constant voltage of 10 kV for 40 min. Absorbance was monitored at 214 nm.

SDS-Capillary Gel Electrophoresis

SDS-Capillary Gel Electrophoresis (SDS-CGE) followed Manso *et al.* (2002), with some modifications. Separations were carried out with a hydrophilic-coated fused-silica capillary CElect 175 (Supelco, Bellefonte, PA, USA) of 0.27 m x 75 µm i.d. (0.20 m to detection point) and eCAP™ SDS 14-200 gel buffer (Beckman Instruments), as electrophoresis buffer. Migrations were run at 25ºC, with a linear voltage gradient of 2-8 kV in 1.7 min, followed at a constant voltage of 8 kV for 15 min. Absorbance was monitored at 214 nm.

Results and discussion

Table 1 shows the nitrogen and protein contents of egg white samples of different poultry species. Protein concentrations ranged for 75 to 85%, except for ostrich, which showed the lowest protein level (64%.)

Table 1. Nitrogen and protein contents of egg white samples from different species. Results are means of two determinations ± SD.

SPECIES	NITROGEN (%)	PROTEIN (%)
HEN	11.97± 0.056	74.81
PHEASANT	12.60± 0.11	78.75
QUAIL	13.36± 0.38	83.50
DUCK	13.60± 0.031	85.00
OSTRICH	10.24± 0.0	64.00

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Figures 1, 2, 3 and 4 show, respectively, RP-HPLC, SDS-PAGE, SDS-CGE and CZE separations of hen egg albumen standards and egg white proteins from different species. The four techniques allowed the separation of the main proteins: ovoalbumin, ovotransferrin, ovomucoid and lysozyme (which constitute 54%, 12%, 11% and 3.4% of hen egg white proteins, respectively, Mine, 1995).

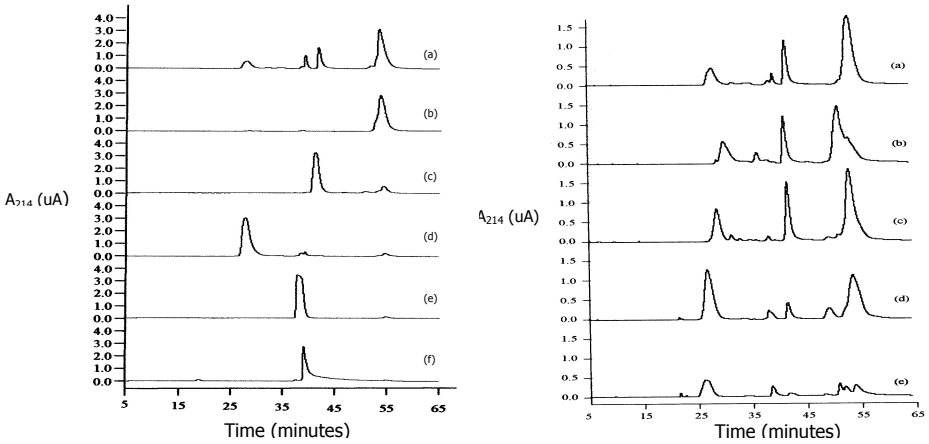

Figure 1. RP-HPLC of standard proteins of chicken egg white **(A)**: a) pooled standard proteins, b) ovalbumin, c) ovotransferrin, d) ovomucoid, e) avidin, f) lysozyme, and egg white proteins of various poultry species **(B)**: a) hen, b) pheasant c) quail, d) duck and e) ostrich.

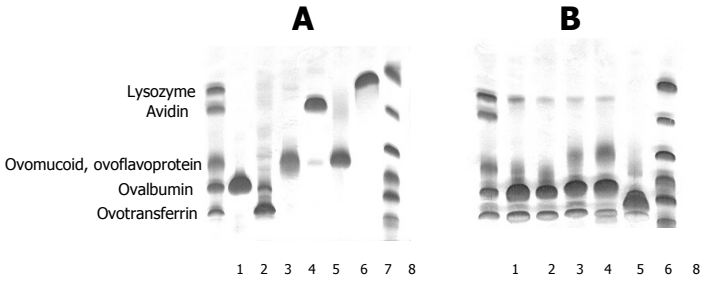

Figure 2. SDS-PAGE of chicken egg white protein standards **(A)**: 1) pooled standard proteins, 2) ovalbumin, 3) ovotransferrin, 4) ovomucoid, 5) avidin, 6) ovoflavoprotein, 7) lysozyme, 8) molecular weight calibration kit (LMWK) and egg white proteins of various poultry species **(B)**: 1) pooled standard proteins, 2) hen, b) pheasant, c) quail, d) duck, e) ostrich. LMWK: (with Mr) phosphorilase b (94,000), bovine serum

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albumin (76,000), ovalbumin (43,000), carbonic anhidrase (30,000), soybean trypsin inhibitor (20,100), α-lactalbumin (14,400).

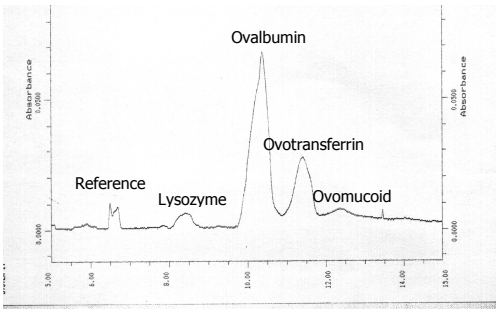

Figure 3. SDS-Capillary Gel Electrophoresis (SDS-CGE) of hen egg white protein.

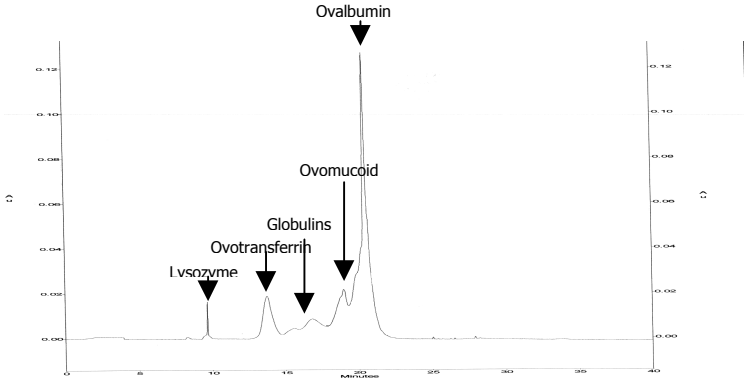

Figure 4. Capillary zone electrophoresis (CZE) of hen egg white proteins.

RP-HPLC proved to be useful for the separation and identification of proteins from different species. Schäfer *et al.* (1995) used a C4 column to quantify albumen proteins during storage of chicken eggs.

As expected, SDS-PAGE showed proteins in a wide range of relative molecular masses, present in very different concentrations (Figure 2). Desert *et al.* (2001) unambiguously identified 6 major proteins in the SDS-PAGE pattern of albumen, however an important number of other well separated bands remained to be characterized. This underlines the complexity of the minor protein components of egg white.

SDS-CGE (Figure 3) affords less resolution than SDS-PAGE (Figure 2) for the separation of egg white proteins, but presents the advantage of providing shorter

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analysis times and thus, it offers the possibility of analyzing more samples in a similar period of time. In fact, Hiidenhovi *et al.* (1995) achieved the separation of the main albumen proteins of chicken eggs in only 7 min by SDS-CGE.
In agreement with Chen *et al.* (1994), CZE provides a well-defined separation pattern (Figure 4). Lysozyme, a very basic protein, shows a short migration time. The use of a high ionic strength buffer minimizes its adsorption to the capillary wall. The other proteins migrate according to their isoelectric points. In addition, as compared with the other separation techniques assayed, CZE affords the separation of the albumen globulins (approximately 8%; Mine, 1995).
Qualitative and quantitative differences were observed among the individual proteins of the animal species investigated. Hen and pheasant, which are very close phylogenetic species (*Phasianidae* family), showed very similar chromatographic and electrophoretic protein profiles (Figures 1 and 2). The duck albumen pattern was characterized for a very high relative concentration of ovomucoid. The ostrich albumen presented the most dissimilar pattern: lysozyme was absent, while ovalbumin presented a very slow migration as compared with the other species, which might be indicative of a higher glycosylation degree.

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CONTENTS

CHARACTERIZATION OF EGGSHELL MICROSTRUCTURE AND ITS INFLUENCE ON MECHANICAL PROPERTIES

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Keywords : microstructure, calcite, optical microscopy, X-ray diffraction.

Abstract

We described different analytical techniques based on optical microscopy and X-ray diffraction to characterize eggshell microstructure, in order to demonstrate the relationship between eggshell micro-structure and its biomechanical properties. Size and orientation of crystals forming the eggshell can be determined directly by optical microscopy on thin-slices observed with an optical microscope with cross-polarizers. X-ray diffraction techniques provide more accurate information about the grain size and orientation. These methods were applied to the changes in eggshell microstructure associated to moulting in order to explain the accompanying changes in the eggshell mechanical properties.

Résumé

Différentes techniques d'analyse de la microstructure de la coquille d'œuf sont décrites afin de déterminer leur effets sur ses propriétés mécaniques. La taille et l'orientation cristalline sont étudiées par microscopie optique sur lames minces en lumière polarisée. La diffraction aux rayons X donne des informations texturales plus précises. A titre d'exemple, nous démontrons que la mue modifie largement la charge de rupture des coquilles sans modifier leur épaisseur et qu'elle est associée à une réduction de la taille des cristaux pouvant être responsable des modifications des propriétés mécaniques.

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Introduction

Eggshell is a bioceramic composite consisting mainly (> 95 %) of the mineral calcite ($CaCO_3$) and a pervading organic matrix (1 - 3.5 %), resulting in a structure which has excellent mechanical properties (Fink et al. 1992). The most important parameter for structural strength of the egg is shell thickness (Tyler 1961). However, considering that the eggshell is composite material made of a mineral part and an organic part, its mechanical properties (breaking strength) should be also determined by mineral part microstructure (defined by the size of crystals, their shape and how are they arranged and oriented in the structure) and the organic matrix (Davidge 1979; Rodriguez-Navarro et al. 2002). We have investigated the changes in eggshell microstructure associated to moulting in order to explain the accompanying changes in the eggshell mechanical properties. To characterize eggshell microstructure, we used different analytical techniques based on optical microscopy and X-ray diffraction. The quantification of some components of organic matrix is described in a companion article (Ayman et al., 2003).

Materials and Methods

a) Crystal size and orientation

1. Optical microscopy

The size and orientation of crystals forming the eggshell can be determined directly by optical microscopy. To study the microstructure of eggshells, we can prepare thin-slices (<30 μm) of radial sections cut normal to the eggshell surface and observe them using an optical microscope with cross-polarizers. Then, average grain size can be assessed by counting the number of grains, *n*, intersecting a line of length, *l* (Dehoff & Rhines, 1968). The orientation of the crystals in the cross-sections of the shells can be determined optically using cross-polarizers, rotating and finding the angle of total extinction for each grain. Although the microstructural information obtained by optical microscopy is very detailed, it is limited to a very small area and to a 2-D section. Then, the information about the grain size and orientation is from a small number of grains and the statistics is poor. Also, in eggshells the differences in extinction does not allow a unambiguous differentiation among grains what makes difficult to count them and even more to automatize this process with a image analysis program.

2. X-ray diffraction

2.a) Grain sizes: X-ray diffraction techniques also provide information about the grain size and orientation. These techniques are more powerful because a diffraction pattern integrates the information from many crystals from a volume of the sample. An x-ray

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diffraction pattern collected with a photographic film or area detector can yield information about the grain size and their orientation (Cullity 1977). When the grain size is quite coarse only a few crystals diffract and only a few spots are registered with the detector. A somewhat finer grain size increases the number of spots. If the grain size is fine enough then a greater number of crystals are illuminated by the beam and a continuous Debey ring is formed. Basically, the number of spots depends on the multiplicity of the reflection and the beam size, which determine the number of grains illuminated by X-rays. Thus, an equation can be derived that relates the observed number of spots on a Debye ring to the grain size previous a calibration with standards of known crystal sizes (determined by optical microscopy).

2.b) Crystal orientation: There are different methods based on X-ray diffraction to measure the orientation of crystals forming an aggregate such as the eggshell. To determine the distribution of orientation of crystals in space, a texture diffractometer is used to register the pole figures. The texture diffractometer have a sample stage holder having two rotational axes, one to spin the sample in a horizontal plane (ϕ angle) and another to tilt the sample (χ angle) from 0 to 85 degrees. To measure the orientation of a set of *[hkl]* crystal directions or *hkl* pole figure in an aggregate, the XRD goniometer is set at a corresponding 2θ angle of the *hkl* reflection and the sample is brought into different orientations by rotating (ϕ) and tilting (χ) the sample holder (Bunge, 1997; Rodriguez-Navarro & Romanek 2002). The intensity of the reflection, recorded at each sample orientation (defined by polar coordinates ϕ and χ), is proportional to the number of irradiated crystals having their *{hkl}* lattice planes in Bragg orientation (Cullity, 1977). Finally, the software plots the pole figures in two dimensions using a sterographic projection. It is also possible to quantify the scattering or degree of preferential orientation of crystals as the breath of the peak distribution displayed in the pole figures, which is measured as the angular width at half maximum of the peaks (FWHM). The sharper the distribution of crystal orientation, the smaller the value of FWHM and the greater is the alignment of crystals in a material.

The full pole figures registered with a texture diffractometer gives a very detail information about the 3-D distribution of crystals in an aggregate. However, it takes a long time to register them and is not practical when analysing a great number of samples. Alternatively, the distribution of orientation of crystal can be determined doing simpler analyses. For instance, when crystals are distributed so that they are preferentially oriented along a crystallographic direction and are uniformly rotated around this axis. Then, the variation of the intensity of the reflection with the tilting of the sample (Psi-scan) describe the distribution of the orientation of crystals. This type of scan is equivalent to do a omega scan using a powder diffractometer. If the diffractometer does not have the possibility of doing omega-scans, the distribution of crystal orientations can be still determined registering a regular θ–2θ scan and measuring intensity of reflections in an oriented samples relative to a randomly oriented sample (Rodríguez-Navarro et al. 2002).

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Results

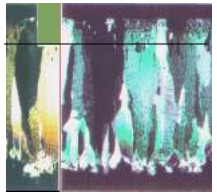
a) Description of optical microscopy and X-ray diffraction techniques for eggshell microstructure characterization:

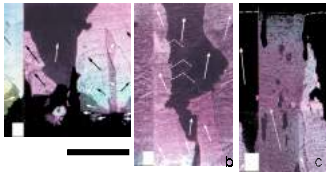
1. Techniques based on optical microscopy

Sample preparation : double polished thin sections (< 30 μm).

Crystal size : esterological techniques ; count the number of crystal grains , *n*, intersecting a line of length , *l*, using a image analysis program .

Average grain size = *l* / *n*.





Crystal orientation : Find the orientation of the crystal axis of each grain by rotating the thin section until the total extintion of each grain under cross-polarized light .

Evaluation : optical microscopy provides a very detailed information about the eggshell microstructure . However , sample preparation is very tedious and is only practical for studiying a few samples . Also, the information is limited to a small number of grains from a 2-D section . Poor statistics . Difficult image analysis .

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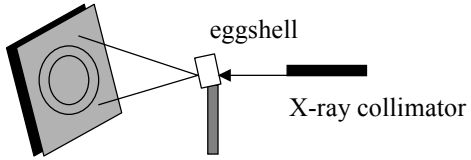
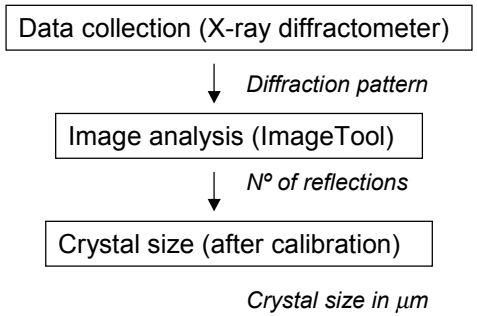
2. Techniques based on X-ray diffraction

Sample preparation: no.

The number of crystals contributing to the diffraction pattern varies with the size of crystals. From size of the beam and the number of reflection spots the size of crystal can be determined. Also, crystal orientation can be determined.



Area detector



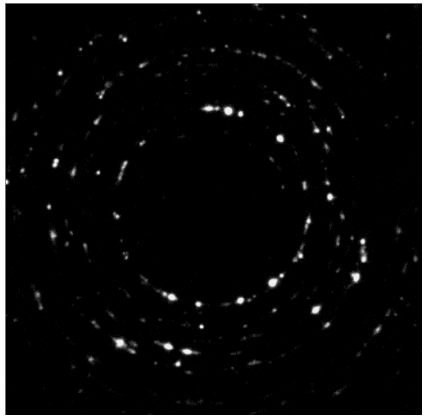


Figure 1. X-ray diffraction pattern from an eggshell

Evaluation: X-ray diffraction integrates the information from all crystals in volume of the sample iluminated by the beam. It provides a better statistics. Also it is faster and easier to analyse and more powerful than techniques based on optical microscopy.

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b) Effect of molting on eggshell microstructure:
In order to understand the improvement of mechanical properties of eggshells after artificial molting (Table 1), we have applied the previous techniques to investigate if there any accompanying changes on eggshell microstructure with this process (Table 2). The results are summarized in the following tables.

Table 1. Effect of molting on eggshell thickness and breaking strength. Eggshell thickness was measured with a micrometer. Breaking strength was measured with an Instron testing machine.

	Before molt	After molt	n	P
Thickness (μm)	371 ± 25	364 ± 31	50	0.15
Breaking strength (N)	30 ± 6	36 ± 7	50	0.00

Table 2. Effect of molting on the size and orientation of crystals composing eggshells. Crystal sizes were determined by optical microscopy (OM) and X-ray diffraction (XRD).

	Before molt	After molt	n	P
Crystal size (OM) (μm)	72 ± 12	58 ± 7	6	0.01
Crystal size (XRD) (μm)	68 ± 9	59 ± 8	24	0.02
FWHM (XRD) (deg)	41 ± 8	43 ± 7	50	0.34

Conclusions

In this paper, we describe the use of optical microscopy and X-ray diffraction to characterize the microstructure of eggshells. These two complementary tools can be used independently or in tandem to characterize size, morphology and orientation of crystal grains composing any mineral aggregate. Optical microscopy provides a very detailed information of the microstructure but require long process sample preparation and is only practical for studying a few samples. On the contrary, X-ray diffraction does not require sample preparation and can be applied for larger number of samples.
There was a significant improvement on the mechanical properties of eggshells after moulting. Interestingly, eggshell thickness did not changed significantly. On the contrary, eggshell microstructure did change. There was a significant decrease in size of crystals making the eggshell, which could explain the improvement on eggshell mechanical properties.

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INVESTIGATION OF BIOMINERALISATION EVENTS: EFFECTS OF PHYSIOLOGICAL STRESSES ON EGGSHELL STRUCTURE USING X-RAY DIFFRACTION AND SMALL ANGLE X-RAY SCATTERING (SAXS)

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Keywords : X-ray diffraction, SAXS, structure.

Abstract

The eggshell is a bioceramic composite in which calcite is the dominant crystal form. Variables such as bird housing, nutrition and environmental stresses can alter the form and texture of the eggshell through alterations in crystal morphology. This paper describes the use of biophysical investigative procedures to resolve different aspects of the shell growth process. 1. X-ray diffraction to analyse crystal size by measuring the broadening of X-ray lines, 2. SAXS to evaluate crystal shape by examining the variations of scattering intensity with regard to the scattering vector q. The preliminary results presented here illustrate how both techniques have been used to investigate the biomineralisation and give an estimation of the size and shape of the crystals within eggshells. Our results indicate that the overall size of the crystals using the FWHM Debye Scherrer method ranges between 54 - 232 nm and between 75 – 106 nm using the Rietveld method. Variations in size may be due to different crystal forms produced during the different stages of eggshell development.

Résumé

La coquille d'oeuf est un biomatériau composite dans lequel la forme cristalline dominante est la calcite. Certaines variables tel que le type d'élevage, l'alimentation ou le stress lié à l'environnement peuvent modifier la forme et la texture des coquilles en altérant la morphologie des cristaux. Cet article décrit des procédures d'investigation biophysiques appliquées à la compréhension de la croissance cristalline des coquilles d'oeufs. 1. Diffraction aux rayons X pour mesurer la taille des cristaux

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par l'analyse de la largeur des rayons X. 2. SAXS pour évaluer la forme des cristaux par l'analyse de la variance de l'intensité dispersée du vecteur 'q'. Les résultats préliminaires obtenus et présentés dans cet article illustrent comment ces deux techniques peuvent être utilisées pour examiner le processus de biominéralisation et pour estimer la taille et la forme des cristaux de calcite de la coquille. Nos résultats indiquent que la taille des cristaux varie de 54 à 232 nm en utilisant la méthode FWHM Debye Scherrer et de 75 à 106 nm avec la méthode Rietveld. Cette variation pourrait être expliquées par la formation de cristaux de différentes tailles pendant les étapes de la croissance de la coquille des oeufs.

Introduction

The eggshell consists of both organic and inorganic constituents. It is made up of approximately 95% calcium carbonate in the form of calcite and approximately 3.5% of proteins and proteoglycans (Arias *et al*., 1993; Gautron *et al*, 2002). These components are thought to influence nucleation and to control crystal growth and shape, and to play a role in controlling the mechanical properties of the resulting composite. It is thought that crystal deposition is controlled by a sequence of proteins secreted in the oviduct that direct crystal formation and shell assembly. Silyn-Roberts and Sharp (1986) suggested that the organic matrix could be acting as a reinforcing fibrous network. As the organic matrix is incorporated into the shell, we plan to investigate further if this will produce different coherent regions and organic/inorganic interfaces.

X-ray diffraction can be used as a means of measuring changes in crystal orientation, size and also any changes in the molecular packing within crystals through examining differences in intensity and position of the diffraction peak profiles. Information regarding crystallite size can be obtained by measuring the breadth of Bragg's peaks, Full Width at Half Maximum (FWHM). When crystallites are less than ~100nm in size, there is found to be measurable broadening in the X-ray diffraction lines. By measuring the FWHM of the diffraction peaks, information on coherent regions of crystallinity can be found. These regions may correspond to the actual size of the crystals or are a measure of coherent regions in the larger particle. An alternative to measuring the FWHM of individual peaks, all the peaks in a profile can be fitted as a whole using the Rietveld method. The Rietveld method is carried out using the least-squares refinements until the best fit is obtained between the entire observed powder diffraction pattern taken as a whole and the entire calculated pattern based on the simultaneously refined models for the crystal structures (Young, 1993).

SAXS is a technique capable of providing information about the size, habit and arrangement of nanocrystals. The intensity distribution of the scattered X-rays gives

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information on average crystal thickness and crystal shape. SAXS and X-ray diffraction differ in the location of the scattering of interest. SAXS is typically at small angles in the vicinity of the primary beam and extends to within 2 degrees for standard wavelengths. In X-ray diffraction, the broadening of X-ray lines from the internal organisation is interpreted compared to SAXS where the broadening of the primary beam is insensitive to the strain and imperfections of the crystallinity, therefore SAXS analysis depends on the outer dimensions of the crystals (Fratzl, *et al*. 1991). SAXS is a useful tool in measuring surface area volume ratio of particles ranging between 0.5 and 50 nm (Glatter and Kratky, 1982).

Material and Methods

Experiments were carried out on stations 2.1 and 14.1 of the CCLRC Daresbury synchrotron. Eggshells from hens where the eggs were expelled at different points of development were used. The eggs were expelled at 8, 10, 12, 14, 16, 18, 20 and 22 hours of development. A pestle and mortar was used to powder the eggshell and the powder was mounted between two mica sheets on a slide.

The FWHM measurements made from the 14.1 data was fitted into the Scherrer equation to obtain particle size

$$D = \frac{K\lambda}{\beta \cos\theta} \qquad \text{where} \quad \beta = \sqrt{(B^2 - b^2)}$$

λ is the wavelength, θ the diffraction angle, K is a numerical constant of the order of unity, taken as 0.94, B is the breadth produced by the small crystallite size, b is the breadth arising from instrumental broadening (Newman, 1999).

The SAXS data obtained at 2.1 were spherically averaged and the two-dimensional images were converted into linear intensity profiles (Fratzl, 1996). The linear SAXS intensity profiles I(q) were converted into Kratky plots ($I(q)q^2$ against q) where $q = 2\pi\sin\theta/\lambda$.

Results and discussion

The diffraction data collected at beamline 14.1 was converted into a linear intensity profile as shown in figure 1. A table of the crystallite sizes, using the FWHM obtained from the [104] orientation peak, is shown in table 1.

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Figure 1: Typical X-ray diffraction line profile obtained from eggshells.

Sample (hours)	Exposure Time (secs)	FWHM (pixels)	Crystallite Size (nm)
8	1	4.1122	54.62
10	1	3.9165	65.38
12	1	3.789	76.57
14	1	3.6794	92.07
16	1	4.0259	58.73
18	1	3.4662	224.54
20	1	3.7755	78.05
22	1	3.4633	232.32

Table 1:Crystal sizes from X-ray diffraction data.

It can be seen from our preliminary results using the broadening of X-ray diffraction lines that the crystal sizes increase up until 14 hours. The 18 and 22 hour results are the only two outwith the rest. Crystal size, strain and imperfection of the crystallinity contribute to the line broadening. The variations in sizes may be affected due to the organic matrix embedded within the crystals and therefore may have reduced the size of the coherent regions.

The measurements made of the eggshell crystals using the Rietveld Method showed that there was little variation in crystal size in relation to time. All measurements gave an average size of about 90 nm, starting with 75 nm at 8 hours, reaching a maximum after 14 – 16 hours of 106 nm and then reducing to 75 nm again at 22 hours. These results show that the crystal sizes are large which supports the results from the X-ray diffraction data. The differences in crystal size obtained using the FWHM Debye

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Scherrer method and the Rietveld method are possibly due to anisotropy in crystal dimensions, however trends are similar. Therefore further investigation using these techniques could be used to measure the effect of organic constituents within the inorganic component of the shell.

The SAXS data collected at beamline 2.1 were converted into Kratky plots shown in figure 2.

$I(q)q^2$

$q\ (nm^{-1})$

Figure 2: Small angle X-ray scattering profile of developing eggshell.

The profiles that are observed from the scattering of eggshell crystals correspond to Lorenzian type curves (Fratzl, 1991). A possible explanation for this is that there is a broad range of sizes and shapes of mineral crystals. Therefore, no clear conclusion can be made of the exact shape of the crystals. Cain and Heyn (1964) have shown that the particle size in eggshells corresponds to 200 microns. However, eggshells have the organic matrix embedded within the calcium carbonate crystals that could affect the size of coherent regions within eggshell crystals. There is some evidence for scattering interfaces of organic material within crystals (data not shown). These regions are ideal for investigating using SAXS to obtain size, orientation and shape of crystals. Further work will include using microfocus SAXS to scan through each of the layers within the eggshell.

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Session 8
Evaluation of egg quality by non destructive methods

NON DESTRUCTIVE MEASUREMENTS OF EGG QUALITY

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Keywords : Egg quality, non-destructive methods.

Abstract

Due to the increasing throughput of modern egg grading machines, which grade up to 120 000 eggs per hour, the visual inspection of eggs by humans ('candling') becomes a serious bottleneck in the egg production chain. In order to assure a high and consistent egg quality, researchers have been investigating the use of modern sensor technologies to replace the candling operation. During the last decades, several types of sensors have been developed, and it is believed that these sensors will replace human candling in the near future. This contribution gives a short overview of these modern sensor technologies for egg grading.

Résumé

En raison des performances croissantes des machines modernes de tri des œufs, qui trient jusqu'à 120 000 oeufs par heure, l'inspection visuelle des oeufs par des humains devient un goulot d'étranglement sérieux dans la chaîne de production d'œufs. Afin d'assurer une qualité des oeufs élevée et fiable, les chercheurs ont étudié l'utilisation des technologies de détecteurs modernes afin de remplacer l'opération de mirage. Pendant les dernières décennies, plusieurs types de sondes ont été développés, et on croit que ces sondes remplaceront le mirage humain dans un proche avenir. Cette contribution donne une courte vue d'ensemble de ces technologies de sonde modernes pour le tri des œufs.

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Introduction

Visual appearance, dirt, cracks, albumen viscosity, yolk colour, internal blood and meat spots and loose air chambers are considered as important egg quality aspects. A number of these quality parameters are routinely monitored in packing houses through candling. However, the increasing throughput of modern egg sorting machines, which can grade up to 120 000 eggs per hour, makes this quality inspection step become more difficult and, hence, less reliable. For other quality aspects, such as eggshell strength or albumen quality, separate techniques, destructive and non-destructive, were developed many years ago. Most of these however have the disadvantage of being time consuming so that they can only be applied on a small sub sample of the considered batch.

Egg quality assessment is not just important to packing houses. Non-destructive (and also destructive) egg quality measurements are also of relevance in the grading of eggs for enhanced safety and quality assurance towards consumers. They can also be of benefit to producers since they gives information on the outcome of the egg production process and hence on the health condition of the flock or on the climatic conditions in the poultry house. Non-destructive measurements of the internal characteristics of eggs can also be used to monitor the incubation process.

The first step towards providing an accurate system for assessing high and consistent egg quality is to develop sensors that are able to control the products. An automated quality evaluation has some advantages compared to a human one: it is more reliable, the productivity may be improved, and machines can "see" more than human beings (for instance "through" the products). New technologies which are fast, completely automated and reliable offer the best possibility for assessing quality in all individual units instead of taking samples. Factors affecting the choice of techniques include consistency within a single product, measurement speed, instrumentation cost and the sorting adequacy required.

The availability of fast computers and new sensor technologies has inspired the research towards such fast, objective and accurate technologies. This text gives a brief review of recently developed techniques for the assessment of several aspects of egg quality.

Mechanical techniques

In general, eggshell strength methods can be subdivided into direct methods and indirect methods (Hamilton, 1982). Different direct methods are described in the literature. Most widely used is the compression fracture force measured during quasi–

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static compression (Voisey and Hunt, 1967; Hamilton, 1982; Abdallah *et al.,* 1993)), which is a measure of its material strength. Other methods include puncture tests and impact tests. All direct methods are destructive. Indirect methods measure a parameter that is related to the eggshell strength. The correlation between the different methods is moderate, and the choice of which method to use often depends on the application. Measuring the eggshell thickness is one of the most frequently applied indirect methods used to give an indication of eggshell strength. Another indirect measure for the strength of the egg is provided by the calculation of the percentage eggshell of an egg. A third widely used indirect method makes use of the quasi–static compression of the eggs between two parallel plates. By measuring the force–deformation curve at non-destructive forces of less than 1kg, it is possible to determine the static stiffness of the egg. The deformation, also known as non–destructive deformation, which can be defined as the amount an eggshell bends or deflects under an applied force, is then used to estimate the force required to fracture the eggshell (Hamilton, 1982) and measures a structural property of the egg. Indirect methods are used to measure eggshell strength on the assumption that the indirect values are correlated with the direct values. However, values found in literature indicate that there are only moderate correlations among those parameters. This observation indicates that there are several mechanisms involved in shell fracture in addition to the strength of the eggshell material.

Mechanical techniques can be used for eggshell crack detection. In two commercial applications a small impactor excites the eggshell. A combination of the amplitude of the rebounds and/or the number of rebounds of the impactor is used as an indication for the local mechanical eggshell integrity. A locally intact eggshell surface allows the impactor to perform several elastic rebounds of high amplitude. In the neighbourhood of a crack, the elasticity of the adjacent shell area is seriously impaired and hence the rebound will be damped heavily. By repeating this action on different places around the eggshell surface, a spatial map of the mechanical state of the eggshell can be built. This is the working principle of the two commercial crack detectors. The role of the impactor can be performed either by a bearing ball built into an electromagnetic probe (Moayeri, 1996) or by the egg itself. In the last case the egg is rolling over a series of small metal objects under which a piezo-sensor is mounted (Bliss, 1973). As this measuring principle reveals only local shell quality information, these crack detectors test several locations for each egg (24 to 32 points per egg) in order to obtain satisfying results. The crack detection rate ranges from 70% to 85% while the percentage falsely rejected intact eggs is between 0.3% and 1% according to the data offered by the manufacturers of the eggshell crack detectors. These values are obtained under practical, industrial conditions on a high volume of eggs.

A different approach was suggested by Coucke (1998). Instead of analysing the behaviour of the impactor after excitation, the response of the egg itself after being impacted was considered. When an egg is subjected to a non-destructive impact

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excitation, the shell will react with an oscillation response. Coucke (1998) and De Ketelaere and De Baerdemaeker (2000) observed that eggs with damaged shells show a greater number of such resonant peaks than intact eggs. They also showed that for intact eggs the impulse response was very similar on every point on the equator, whereas eggs with a damaged shell show a different response on different locations of the equator. This finding led to the construction of a crack detection algorithm which is based on the correlations between repeated measurements taken on the same egg. Only 4 measurements for each egg are needed. The researchers showed that in this way up to 90 % of the cracks can be removed, while the false rejects remain below 1 %. The fast nature of the technique, an egg vibration typically lasts for about 10 ms, and the low number of measurements needed, make this technique a very appealing alternative to the principle applied in current crack detectors.

The same authors (Coucke, 1998; Coucke et al., 1999; De Ketelaere and De Baerdemaeker, 2000; De Ketelaere, 2002) applied their acoustic test to estimate eggshell strength of intact eggs. For this purpose, Coucke (1998) modelled the egg as a mass–spring system and defined a novel eggshell strength parameter named 'dynamic stiffness'. The term dynamic was chosen in order to highlight that the shell strength was measured using dynamic measurements, i.e. after being impacted. Coucke (1998) and Coucke et al. (1999) reported a correlation of 0.71 between the static and dynamic stiffness. The correlation between the eggshell thickness and the dynamic stiffness was 0.60. Later, De Ketelaere and De Baerdemaeker (2000) and De Ketelaere (2002) extended the initial mass–spring model towards a mass–spring–damper model and showed that the damping of the vibration, although these researchers at this moment do not have a clear view of its interpretation, provides extra information linking the dynamic measurements to static measurements. Furthermore they showed that the shape of the egg is needed to link dynamic to static measurements. A multiple linear regression model using the dynamic stiffness, the damping and the shape index of the eggs as explanatory variables in order to predict the static stiffness with a correlation of 0.90 was constructed. The same explanatory variables were used to estimate shell thickness and breaking strength, but correlations were lower (0.78 and 0.64, respectively).

In another application of the same technique, Coucke (1998) demonstrated that the course of the vibration behaviour of fertile incubation eggs suddenly changes around 100 hours of incubation while infertile eggs did not show this behaviour. Bamelis (1999) showed that this sudden change was due to the formation of sub embryonic fluid. De Ketelaere (2002) and Kemps et al. (in press) remarked that the moment of change is related to the moment of hatch and, hence, to embryonic growth speed.

Thus this type of indirect measure could be potentially applicable for use not only in packing houses, but could also be used to monitor incubation processes, and the health and welfare of birds. In this context, Bamelis et al. (2002a) showed that heat stress

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could be detected by monitoring the dynamic stiffness of eggs during the laying period.

Spectroscopic techniques

Near Infrared Spectroscopy (NIR) is a technique which has been readily used for determination of the internal quality of agricultural products (Williams and Norris, 1987). Analysis of NIR absorption spectra results in quantitative information about constituents like, for example water and proteins (Giangiacomo and Dull, 1986). NIR measurements have several advantages; they are quick, non-destructive, accurate, reliable, contactless and economical. The contactless nature of the technique makes it very appealing to score egg quality parameters since contact has to be minimized from a hygienic viewpoint and large amounts of eggs have to be graded.
Often, no sample preparation is required. NIR works by measuring the vibrations caused by the stretching and bending of hydrogen bonds with carbon, oxygen and nitrogen. The utilisation of these optical techniques with regard to quality determination of eggs is however rather limited. Norris (1996) published an article concerning NIR measurements for freshness detection in shell eggs. He studied the effect of egg age on its optical properties. He saw a very definite time related change in the spectra extending over several hours right after laying. However, it was not a meaningful change with regard to egg quality. It seemed that the change in scatter properties of the eggshell was measured as the moisture content of the shell stabilizes during the first hours after laying. Kemps et al. (unpublished results) showed that NIR spectral analysis of albumen might reveal interesting information relating to egg freshness. Based on three different ratios (T442 nm / T470 nm, T649 nm /T 667 nm and T1084 nm / T1286 nm) this author showed that a prediction of the Haugh units of an individual egg is plausible using a single measurement. A correlation of 0.7 was obtained between the measured and the predicted Haugh units. For albumen quality, the main reference point has mainly been Haugh unit (Haugh, 1937) for which the egg is broken and the height of the thick albumen is measured and corrected for egg mass. It might therefore be interesting to link optical or other non-destructive physical measurements to a more physical albumen quality parameter rather than to the intuitive Haugh unit. At present, only the work Schmilovitch et al. (2002) has attempted to do this. These researchers showed that pH could be determined using a Partial Least Squares regression model on the first derivative of the spectral data. To date there are no references available linking albumen viscosity, as another important physical quality attribute, to spectral data. Moreover, the literature on albumen viscosity measurements is limited, probably due to the Haugh alternative, and due to

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the complexity of measuring albumen viscosity using the rheological approach (Tung et al., 1970; Pitsilis et al., 1984; Lucisano et al., 1996).

A practical application of NIR measurements has been found in relation to the 'enriched eggs' market. Egg producers are now enhancing their products with supplements such as DHA, LNA, and Vitamin E. Based on NIR technology producers can ensure their eggs do contain these ingredients (Anonymous, 1). There is also interest for analysing the amount of dissolved CO in the albumen. Because of the viscous nature of egg components, direct measurements using a CO-ion select probe would not adequately measure dissolved CO-levels. Calibrated NIR systems and gas chromatography systems are available for CO-analysis. However, these are expensive and require periodic calibration (Anonymous, 2).

Mielke et al. (2002) investigated the use of NIR-spectrometry of egg products. Genetic origin, storage conditions, age of hens and carotenoids in chicken feed were taken into account in their experiments as well as pasteurisation for their possible influence on spectral properties. Rapid NIRS methods were established for analysis of various egg components, e.g. dry matter, protein, fat, lecithine, and cholesterol. Performance proved comparable to standard lab methods.

Optical techniques are also used to check for the presence of pigments in eggs. For the quality control of consumption eggs, two pigment-controlling applications can be found: those applications which search for eggshell pigmentation or shell colour, and secondly applications which search for pigments inside the egg, particularly in the detection of blood and / or meat spots in the egg. Although eggshell colour doesn't have any influence on the nutritional value of the egg for humans, it has an important influence on consumer preferences, making the colour of the eggshell an important economic quality parameter (Wei and Bitgood, 1989). Eggshell colour is mainly determined by the presence of the pigment protoporphyrin in the eggshell (Lang and Wells, 1987), but also another pigment called billiverdin contributes to the eggshell colour as experienced by the human eye (Kennedy and Vevers, 1973). Both pigments have different absorption peaks and so eggshell colour cannot be determined by measuring light at one or two specific wavelengths. On the other hand, pigments present inside some eggs, do not influence the egg colour. For this reason, eggshell colour measuring applications always measure the reflected light and not the light that is transmitted through the egg. Eggshell colour is therefore measured by the reflected light at three specific wavelengths, being the characteristic wavelengths of red, green and blue light. These three values are combined to calculate a specific value depending on the type of the application, which provides information about the colour of the eggshell (Wei and Bitgood, 1989). Automated colour grading techniques are not installed on large scale-grading machines, but they are used in layer lines selection centres.

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When measuring transmission spectra of eggs, light travels through the egg and the absence or presence of internal optical active abnormalities can be detected. These abnormalities are, for instance, blood and meat spots. The detection of meat spots is very difficult and not used in practice yet. Contrary, the detection of blood in eggs is used worldwide in grading machines. The optically active pigment of blood, haemoglobin, has three absorption peaks, namely at 415, 541 and 577 nm. As the calciferous shell of the egg is absorbing all transmitted light beneath 500 nm, only the 577 nm absorption peak can be used to detect the presence of blood in eggs (Brant et al., 1953). To correct for eggshell thickness, eggs size and other non hemoglobin–dependent variables, this 577 nm value is divided by a reference value, depending on the author between 585 and 610 nm (Gielen et al., 1979). The ratio between the two transmission values is called the 'blood value' and is used as rejection criterion. Brant et al. (1953) mentioned the fact that large bloodspots and bloody white can be detected with this technique, but smaller bloodspots remain hidden.

Our own research pointed out that a very small amount of blood in the albumen can be detected but only when part of it is dispersed in the albumen. However, very small bloodspots are often not associated with a zone of dispersed blood and will not be detected. On the other hand, a small amount of dispersed blood without the presence of a bloodspot cannot be seen with the human eye, but is recognised by the detecting mechanism and these are often thought to be false rejects. The brown pigment of the eggshell, protoporphyrin has an adsorption peak at 589 nm, very close to the absorption peak of hemoglobin, making the detection of blood in brown shelled eggs particularly difficult. Even the use of a ratio of two wavelengths in the blood value cannot solve this problem (Gielen et al, 1979). Some detectors can adapt the selection threshold based on a colour measurement of the eggshell, but the detection still remains difficult.

The presence of blood in consumption eggs is a negative quality characteristic, however in hatchery eggs, this characteristic indicates the growth of an embryo inside. Das and Evans (1992a) describe a system using a machine vision technique for distinguishing between fertilised and non-fertilised eggs. First the eggs are highlighted by a candling system and an image filtered with a 575 nm band pass filter of the egg is then analyzed. Gray levels present in the image are subsequently counted. In fertilized eggs the lower grey levels are more abundant. These lower grey levels are located near the growing embryo, where the 575 nm light is absorbed by the initial blood formation. Using this machine vision technique, the distinction between fertile and infertile eggs can be made with accuracies of 96 to 100% at day four and with 88 to 90% at day three of the incubation. When using a neural network classification system, even at day two only a 67% accuracy can be achieved according to Das and Evans (1992b). As this system has only been tested on white-shelled eggs, it is not known whether it can be used to check brown eggs for fertility. The calculation of the blood value as it is used in grading table eggs and described before can however be used to

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detect blood in the fertile brown hatching egg. The blood value of both white and brown shelled eggs decreases after 72 hours of incubation (Bamelis et al, 2002b). Since the first hemoglobin pigments are synthesised on the surface of the yolk and no light can pass the yolk, it is particularly difficult to detect blood from the first moment it is formed, although it could be present in relatively high amounts. Only later on, when the yolk becomes more translucent by the development of sub embryonic fluid, can the hemoglobin alter the transmitting light enough to be detected. Detection of blood in hatching eggs to check for fertility is not used in industry, but could be a powerful tool in research programs, since the point in time for the first detection of blood could give information about the growing speed of the embryo very early on in the incubation process.
Schwägele et al. (2001) used low resolution H-NMR spectroscopy as a possible non-destructive method to determine the quality of intact eggs. The longitudinal and transversal relaxation times give information on the chemical-physical changes of the specimen under study and, hence, could be used to assess egg freshness. Changes in the transversal relaxation times during storage were reported by these researchers. These changes were found to be due to the increasing liquefaction of albumen during storage.

Camera inspection systems

Camera inspection systems have been used by several authors for the assessment of different aspects of egg quality. Elster and Goodrum (1991) developed a program to analyze grey-scale images of stationery eggs for cracks. The egg was isolated from background noise and enhanced using image processing algorithms. A 96% success rate was achieved. However, the average time required to process one egg was 25.3 seconds. Goodrum and Elster (1992) extended their work to detect cracks at any point on the surface of rotating eggs. The identification of cracks was dependent on the egg size and required software calibration constants. Patel et al. (1994) used image acquisition routines from the work of Elster and Goodrum to capture grey-scale images of cracked and grade A eggs. Histograms of the images were generated and used to train a neural network for the detection of cracked eggs. The model was 90% accurate and provided a significant improvement in speed over the method of Elster and Goodrum. The work was extended to the detection of blood spots and dirt stains (Patel et al., 1996). The neural network model for blood spot detection was 85.6% accurate. An accuracy of 80% was achieved on dirt stain detection. Later, Patel et al. (1998) used color image analysis for the same purpose. Their neural network model for blood spot detection had an average accuracy of 92.8% (90.0% on blood spot eggs and 95.6% on eggs without blood spots). The neural network model for dirt stained

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eggs had an average accuracy of 85.0% (85.6% on eggs with dirt stains and 84.4% on eggs without dirt stains). The average accuracy of the crack detection neural network was 87.8% (84.4% on eggs with cracks and 91.1% on eggs without cracks). Similar research has been performed by Garcia-Alegre et al. (1997) and Feyaerts et al. (1999). Kuchida et al. (1999) used colour image analysis to estimate the yolk–albumen ratio. They report a correlation of 0.83 between the yolk–albumen ratio and two parameters extracted from the image.

Other techniques

Besides higher–mentioned novel techniques, still other types of measurements have been investigated. Voisey and Hamilton (1976) used ultrasonic equipment to measure eggshell thickness and reported that the correlation coefficient (r) between observed and predicted values was 0.74. This principle is nowadays commercially available.
Hutchison et al. (1992) examined the inner structure of the egg using magnetic resonance imaging (MRI) and concluded that MRI could be used successfully in assessing the microanatomy of eggs. However, the inspection of eggs using MRI is impractical, because the equipment is very expensive and not yet in popular use.
Very recent research (Dutta et al., 2003) shows that an electronic nose based system, which employs an array of four commercial tin-oxide odour sensors, can be used to analyse the state of freshness of eggs. They applied a wide range of multivariate statistical techniques to define regions of clustering in the multisensor space according to the state of freshness of the eggs. The results suggest that it is possible to classify eggs into three different freshness stages with up to 95 % accuracy. It is not clear what chemical compounds are involved in this type of freshness detection.

Conclusions and perspectives

The demand in fast, non-destructive and reliable egg quality assessment techniques has resulted in the development of several principles. Most of these principles, such as the acoustic or optical method show promising results in lab-scale experiments, but commercial applications have not emerged yet. One of the reasons is the fact that classic methods such as measuring Haugh units or shell breaking force date back over 50 years and people are used to their use and values. Terms such as the dynamic stiffness of an egg or optical absorption at a given wavelength are less widely publicised and so people hesitate to use these terms. However, it might not be good practice to try to mimic older destructive methods such as breaking strength with these

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new techniques and to report results in terms of the older standard units, since it is generally known that these old reference methods have their own deficiencies, making them unsuitable as a standard reference.

A fast and non-destuctive quality assessment tool together with the modern information technology has multiple advantages that reach far beyond the classical grading step in the overall egg production chain. In the packing house, such fast technologies allow for measuring the quality of an individual egg instead of estimating the quality by sub-sampling some eggs out of a batch. This allows for very concise and fast tracking of the batch quality. Moreover, these techniques allow for measuring more quality attributes and thus can guarantee a better overall quality of each individual egg. The data that is gathered from packing houses can also be fed back towards egg producers by means of the modern information technologies. This allows the producer to track the quality of the delivered eggs, and eventually to trace abnormalities within hours. As an example it is proven that the dynamic stiffness of an egg is impaired by heat stress. By monitoring the dynamic stiffness, a measurement that takes only milliseconds, the producer himself using an off-line unit or from data received from the packing house, can obtain useful and quick information on the status of his flock. As such, non-destructive measurements could therefore equally be regarded as being an important management tool.

Although promising, these new technologies still have to prove their merit in practice. Large pilot studies under commercially relevant conditions are needed in order to judge their real potential and economical value. Once the outcome of these studies proves their added value, it is believed that the newly developed techniques might replace the older standard techniques in the future. However, this process may take a substantial amount of time in order to make people used to their principles and units.

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NON-DESTRUCTIVE INTERNAL EGG FRESHNESS ASSESSMENT USING VIS-NIR SPECTROSCOPY

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Keywords : Egg quality, optical measurement technique, egg freshness

Abstract

Egg freshness is an important quality-property of both consumption and hatching eggs. Nowadays, the freshness of the egg is measured by calculating the Haugh-units of the egg white. Since this technique is destructive, it can only be done on a small sample from a batch of eggs. Optical measurement techniques are fast and non-contact. VIS/NIR light can pass through the shell so that information about of the inside of the egg can be obtained.
In this research, the changing optical properties of eggs are measured using transmission light in the VIS/NIR range over a period of 21 days of storage. A quadratic decrease with time of the amplitude at 663 nm is found. This decrease can be measured by the ratio of amplitudes at 674 and 663 nm (R^2=0.9611).

Résumé

La fraîcheur d'oeufs est un critère important de qualité pour les oeufs à couver et de consommation. Jusqu'à aujourd'hui, la fraîcheur des oeufs était mesurée en calculant les unites Haugh du blanc d'oeuf. Cette technique est destructive et ne peut être faite que sur un petit échantillon de blanc. Les techniques de mesure optiques sont rapides et sans contact direct. En outre, la lumière infrarouge VIS/NIR peut traverser la coquille et donner des informations sur le contenu interne de l'oeuf sans le casser. Nous avons enregistré les modifications des propriétés optiques des oeufs, mesurées en lumière infrarouge (VIS/NIR) en transmission, pendant une période de 21 jours de stockage. Une diminution quadratique d'amplitude à 663 nm a été observée. Cette diminution peut être évaluée par le rapport des amplitudes à 674 et à 663 nm (R^2=0.9611).

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Introduction

Besides food safety, food quality is becoming one of the most important concerns in food production. The quality of the egg white is thought to be strongly related with egg freshness (Silversides and Scott, 2001). The latter is commonly determined by measuring the Haugh-units of the egg white. This is a destructive test that can only be done on small samples of all batches commercialised daily.
Haugh-units, and thus egg freshness, are known to change with the age of the egg. Haugh units are also influenced by the age and the breed of the layer flock, storage conditions and shell properties. This means that the age of the egg cannot be used as a rigid system to measure the egg freshness (Williams, 1992).
In order to guarantee a certain freshness of all eggs in a certain batch, a non-destructive and fast measurement technique has to be found. Optical measurement techniques are often used for this purpose. Since these types of measurements are very fast, non-contact and so non-destructive, they show promise for use in food-applications. Moreover, a large part of the spectral range in the visible and near-infrared spectrum can pass through the eggshell, (Bamelis *et al.*, 2002) and hence provide information on the internal properties of the egg, in particular the egg white. Light does not pass through the yolk (Williams and Norris, 1987)
In the work presented herein, the possibilities of determining internal egg freshness by measuring light transmission through the egg are tested.

Material and Methods

147 uncracked white-shelled eggs (Lohmann, Nijs, Dessel, Belgium) were used in the experiment. All eggs were stored for 21 days in a climatised room at 18°C. Except during the weekends, a transmission spectrum was taken daily, for a total of 15 spectra from each egg.
During the measurement, the egg was placed with its blunt end uppermost on a foam ring, in which a collimating lens was attached. From this lens, an optical fibre transported the light in an Avaspec-2048 spectrophotmeter (Avantes, Eerbeek, The Netherlands). A halogen lamp was placed above the egg. The spectrum of the lamp is uniform with a range of500 - 880 nm. Before and after the measurements, the egg was protected from light and from the lamp heat by a metal lid, which is placed between the lamp and the egg.
All measurements were compared to a reference source. Teflon-block and electrical noise was subtracted to exclude spectral changes in the lamp during the 21-days of the experiment. An integration time of 250ms was used and each spectrum was an average

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of two measured spectra. A Labview-program was written to coordinate the measurements.
Eggshell thickness and egg size are known to affect light transmission through the egg. In order to correct for these factors, normalised transmission spectra were calculated by dividing the measured transmission spectra by the norm of the spectrum. As a result, the surface beneath the measured spectrum is constant for all spectra.
From these normalised spectra, a daily mean spectrum was calculated, providing a set of 15 mean-normalised spectra which were used for statistical analysis.
First, the amplitude of each wavelength was fitted to the day of storage in a linear and quadratic way. R-square and the P-value were calculated for each fit and the best fit was taken out. This is called the amplitude-fit.
Second, the ratio between each wavelength present in the spectrum was fitted for the whole set of spectra and these values were fitted to the day of storage, linearly as well as quadratically, and the R-square and P-value for each fit was calculated. These results were presented in a 3-dimensional plot to provide a zone of maximal correlation of the spectra. This plot was a useful tool to determine two wavelengths which could be used to model the evolution of optical properties of egg white.
All these calculations were done with Matlab 5.3 software.

Results and discussion

Figure 1 shows the mean normalised spectra. They appear to be only different in a small range between 630 and 700 nm, as shown in the detail.
When fitting the amplitudes to the days of storage in the mean spectra, the highest R-square scores and the lowest P-values were achieved with a quadratic fit. A zone of highly significant fits was achieved between 640 and 750 nm. When looking at the spectra, this was the range at which the maximum transmission amplitude was achieved. As an R^2 value of 0.60 is not extremely high, further processing was needed.
In figure 2, the P-value of the fit between the storage time and all possible ratios in the spectra are presented. The results from the quadratic fit are presented, since these show the highest significance. In figure 3, the R-square values for the same fits are presented.

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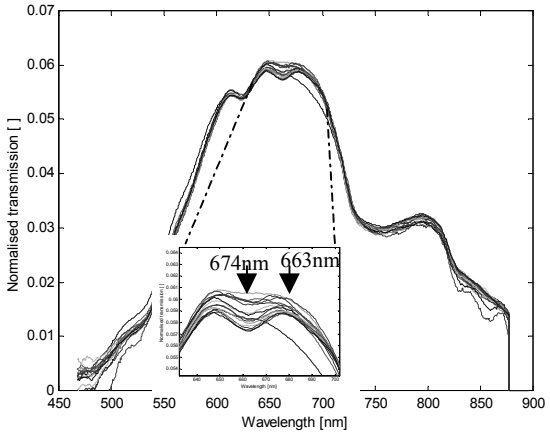

Figure 1*: Mean normalised spectra, one each day. The detail presents the range of the spectra between 630 and 700 nm.*

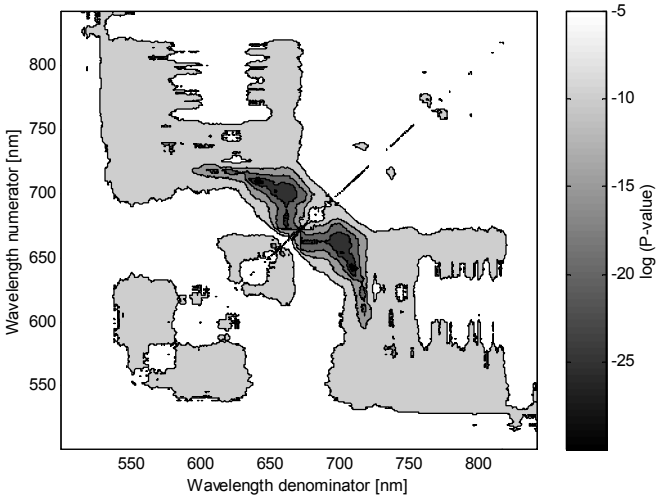

Figure 2: *Log (P-values) for all models in which the storage time is quadratically fit with the ratio between two wavelengths.*

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Figure 3: *R^2 values for all models in which the storage time is quadratically fit with the ratio between two wavelengths*.

In a further step, ratio's of transmission values at different wavelengths were selected to relate them to the egg storage time. Here, a highly significant zone (R^2>0.9) can be noticed in the same zone as for a simple amplitude fit. The ratios with 660nm as the denominator and amplitudes in the range 665 - 740 nm as numerator were most promising.
In figure 1, a detail of all mean spectra is presented around the zone of interest. It can be seen that a decrease is present in spectra around the 660nm. A model was built with the ratio 674nm/663nm. 674 nm was chosen because at that point the normalised mean spectrum is increasing with storage time. 663nm was chosen because a decrease was observed at this point. In fact, the ratio 674/663 is a measure for the decrease which appears when the storage time increases. Again, the quadratic model showed the highest significance and is presented in figure 4.
This model is highly significant (R^2=0.6911 and P<0.0001) and it can be concluded that when egg storage time increases, an absorption peak emerges and the height of the absorption peak increases quadratically in time.
So, the size of the absorption peak is a measure of the age of the egg. This technique could be used in a non-destructive and fast measurement technique.
Absorption peaks in spectra are caused by chemical substances with an optical activity like proteins (Williams and Norris, 1987). At this moment, it is not yet clear which protein causes the spectrum of eggs to decrease at 660nm when the egg is stored for a longer period. But, it can be said that it has to be a protein which is synthesised or released in the egg white during the storage of the egg.

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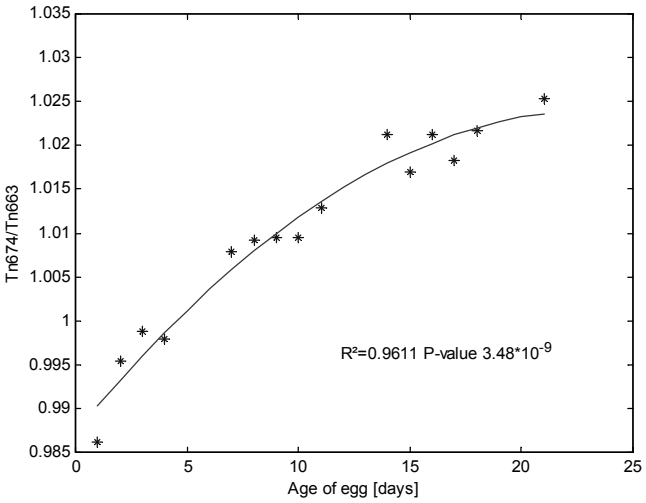

Figure 4: *Quadratic model between the storage time and the size of the decrease, given by the 674/663 nm ratio. Stars indicate the daily mean 674/663nm ratio.*

Conclusion

An optical measurement system and data analyse procedure is presented for non-destructive monitoring of egg freshness. It appears that in longer stored eggs, an unknown substance, probably a protein, is causing a decrease in transmission at 663nm. The size of this decrease, which can be measured by the ratio of transmission values at 674 and 663 nm, is quadratically related with the storage time. It could be the basis of a non-destructive and fast measurement technique for the age of the eggs.

Acknowledgements

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DETECTION OF DIRT ON EGGSHELLS BY MEANS OF A CAMERA INSPECTION SYSTEM

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Keywords : camera inspection, dirt detection, eggshell

Abstract

An automated device for capturing and analysing images was constructed. The set up consists of a digital camera (Sony DFW-VL500) connected to a computer using a Fire Wire link. The egg is placed on two rubber rollers. Two tube shaped lamps (6 W each) are placed on either side of the egg parallel to its longitudinal axe. Two light bulbs (60 W each) are placed at the two poles of the egg. Every picture consists of 640 x 480 pixels. Each image is processed in an identical way. First the whole egg is isolated from its background. This results in a binary image in which the whole egg, regardless of having stains, is white and the background is black. In a second step the egg is isolated from the stains on the shell and background. In this binary image the egg surface is white and the stains and background are black. Finally the number of white pixels in both images are calculated and compared. If the difference between both images is higher than 2 % the egg is regarded as being dirty.

Résumé

Un système automatique de capture et d'analyse d'image d'œuf est décrit. L'installation se compose d'un appareil-photo numérique (Sony DFW-VL500) relié à un ordinateur en utilisant un lien 'Fire Wire'. L'œuf est placé sur deux rouleaux en caoutchouc. Deux lampes en forme de tube (6 W chacune) sont placées de chaque côté de l'œuf, parallèles à son axe longitudinal. Deux ampoules (60 W chacune) sont placées aux deux pôles de l'œuf. Chaque image se compose de 640 x 480 pixels et est traitée d'une manière identique. D'abord l'œuf entier est isolé de son fond. Ceci a comme conséquence une image binaire dans laquelle l'œuf entier, indépendamment

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d'avoir des taches, est blanc et le fond est noir. Dans une deuxième étape l'œuf est isolé des taches sur la coquille et du fond. Dans cette image binaire la surface des œufs est blanche et les taches et le fond sont noirs. Les nombres de pixels blancs dans les deux images sont calculés et enfin comparés. Si la différence entre les deux images est supérieure à 2 % l'œuf est considéré comme étant sale.

Introduction

The market of fresh table eggs has reached its limits of consumption and profit margins are low. Producers need to take care that their products are of high quality. Eggs can be regarded as pre-packaged food with the shell serving as it's wrapping. As no store will ever allow their goods to be on display in a smudged wrapping, eggshells need to be clean. The microbiological quality of the eggs is one of the main considerations of egg quality as it directly determines the shelf life and safety of the product. To ensure the microbiological quality of the egg, shells cannot display blood and faecal stains. For this reason both the USA and the E.U. have developed two different policies. The USA poultry industry is allowed to wash its table eggs, whereas this is prohibited in the E.U.

Shells can be stained with faeces, urine, blood, feathers, egg yolk and egg white. The inspection and grading of table eggs on their shell quality is the only process in egg handling that has not completely been mechanised. This quality check is performed manually by 'graders'. This is not ideal since the accuracy of this process depends on human subjectivity and is often accompanied by visual stress and tiredness. Therefore, automated inspection systems could be very helpful, and several researchers have investigated their use, but such systems have not yet reached the commercial market (Garcia-Allegre et al., 1997 and 2000).

The present work is focused on designing an off-line camera inspection unit that can detect stains on the eggshell and classify eggs into one of two categories: dirty and clean.

Material and Methods

The set-up used to capture and analyse the images is displayed in Figure 1. The camera used in this test set up is a Sony DFW-VL500 with a resolution of 640 x 480 pixels, 32 bits RGB. The output signal of the camera is digital IEEE 1394. The camera was connected to a laptop (Pentium, 233 Mhz).

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Because of the curvature of the eggshell surface, it is difficult to get a uniform lighting without creating shades at the two poles of the egg. Several tests were therefore performed with different lighting settings to get a uniform illumination of the egg. The best results were obtained by using two tube shaped lamps (6 W) placed along side the egg parallel to its longitudinal axis. Two light bulbs (60 W) were placed at each pole of the egg. The two tube lamps needed to be placed very close to the egg, which resulted in the two casings blocking a part of the egg. In a later version of this apparatus it is proposed that the egg will be turning around its longitudinal axis ensuring a complete inspecting of its surface. This will simplify the distinction of the visible part of the egg from the background.

Eighty dirty, brown eggs which were representative of the full range of possible shell stains and sixty clean, brown eggs with shell colour varying from light to dark brown were photographed with the set-up shown in Figure 1. The eggs were placed with their stains towards the camera. The images were stored on the laptop and were then processed using "Imaq TM Vision Builder 5.0" (National Instruments). This program allows the pictures to be manually processed and saved at every step in a script. This made it possible to test if the used detection criteria for dirt on the shell didn't result in creating false positives in the dataset associated with the clean eggs. This script was then used to create a program in Labview (National Instruments®).

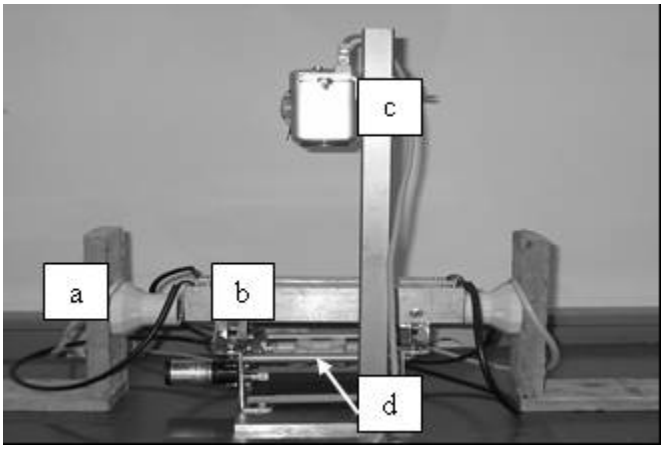

Figure 1: Side view of the set-up to capture and analyse the images. (a): light bulb; (b): casing tube shaped lamp; (c): camera; (d): egg support.

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Image processing algorithm

General

The program starts with calculating the total number of pixels of the visible part of the eggshell regardless of the egg having stains or being clean. Stains on the shell are removed by processing the image. This results in a binary image in which the visible part of the eggshell is white and the background is black. The white pixels are then counted and this number is saved. The image is then processed to detect stains on the shell, which produces a binary image in which the background and the stains are black and the eggshell white. The white pixels in this image are counted and compared with the previous value. When the difference between the two values is more than 2% the egg is classified as being dirty. This threshold of 2% is necessary as during the different processing steps some of the pixels at the edge of the eggshell surface disappear into the background, therefore clean eggs will also display a small difference between their two values.

1. Calculating the pixels of the eggshell surface

A mask is placed over the picture in order to remove the rubber egg support from the image. This image is used as starting point in the next sections of the program. The red colour plane is isolated and a set of grey values is selected: 136 – 255. This transforms the picture to a binary image. Small particles in the background are removed and the holes (black sections) in the remaining particle (the eggshell surface) are filled. A convex envelope is then calculated for that particle in order to fill holes that were in contact with the image border. Finally an erosion is applied. This last operation decreases the shell area on the image. This step is necessary to avoid clean eggs reaching the threshold limit as this step is also used in the stain detecting part of the program.

2. Detection of dark stains, part 1

This string of image process steps starts with the masked image from the previous section. The blue colour plane is isolated and an erosion is applied. Next a set of grey values is selected to transform the image to a binary image. This selection is based on the eggs colour. A region of interest (ROI) can be defined by using the binary image of section 1 as a mask on the blue colour plane. In this ROI the number of pixels with grey value 50 are counted. On the basis of this number different sets of grey values can be selected. In the resulting image the selected stains together with the background will be black while the surface of the eggshell will be white.

3. Detection of dark stains, part 2

The red colour plane is isolated from the masked image of section 1 and an erosion is applied. A set of grey values is selected to transform the image to a binary image. These possible selections are also dependent on the number of pixels with grey value 50 calculated in section 2. Because these selections start with grey value 0, the

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background will be included resulting in a binary image in which the stain and the background are white and the shell surface is black. In the next step the background is removed resulting in a binary image in which only the selected parts of the stains are white. Since only a small part of the stain is selected, in order not to select any clean parts of the egg, a dilatation is applied to make the particles bigger. Finally a XOR operation is performed with the end image of section 1 resulting in a binary image in which background and stains are black and egg surface is white.

4. Detection of dark stains, part 3

This section is basically identical to the previous one apart from the fact that the blue colour plane is used as starting point and different sets of grey values are selected.

5. Detection of white stains

The blue colour plane is selected from the masked image. The brightness and the contrast of the image are adapted. A ROI is defined by using the binary image of section 1 and the pixels having a grey value of 49 and 109 are calculated. On the basis of these two numbers a set of grey values is selected. In the resulting binary image the stains are white whereas the background and the eggshell are black. A dilatation ensures that the particles increase in size. By performing a XOR operation with the end image of section 1 the stains and background are black and the eggshell white.

6. Adding and comparing pixel counts

The 4 images obtained in section 2 to 5 are added by using an AND operator and the number of white pixels of the resulting image is calculated. This value is compared with the number of white pixels counted in section 1. If the difference is bigger than the threshold of 2% the egg is considered dirty. This threshold is set to 2% because some of the image processing steps, e.g. the convex operation in section 1, alter the number of pixels of the eggshell surface.

Results and discussion

A data set of 500 clean eggs and 500 stained eggs was created by visually classifying the eggs. A first draft of the program (without part 2 and 3 for detecting dark stains) was tested on this data set. This first test resulted in no clean eggs being classified as stained though 18% of the eggs with stains were not detected.

A second test with the full version of the program resulted in 0.6 % of the clean eggs being misclassified whereas the number of detected dirty eggs was increased to 90 %. Most of the non-detected eggs had a small yolk or egg white stains or light coloured bloodstains. Since the two light bulbs were placed close to the egg the detection method for white stains can detect large stains of egg white since they appear on the blue colour plane as white stains. Further work is being done on simplifying the program and adapting the set up to rotate eggs.

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Conclusions

The present work concentrated on creating an off-line unit to detect dirt on eggshells. The classification was based on calculating the surface area of the visible part of the egg and comparing it with the surface area minus the stains. The set up was tested on 1000 eggs and a detection of 90 % was reached where only 0.6% of the clean eggs were wrongly classified as being dirty.

Acknowledgements

T. Govaerts is supported by the Ministry of Small Enterprises, Traders and Agriculture-Research.

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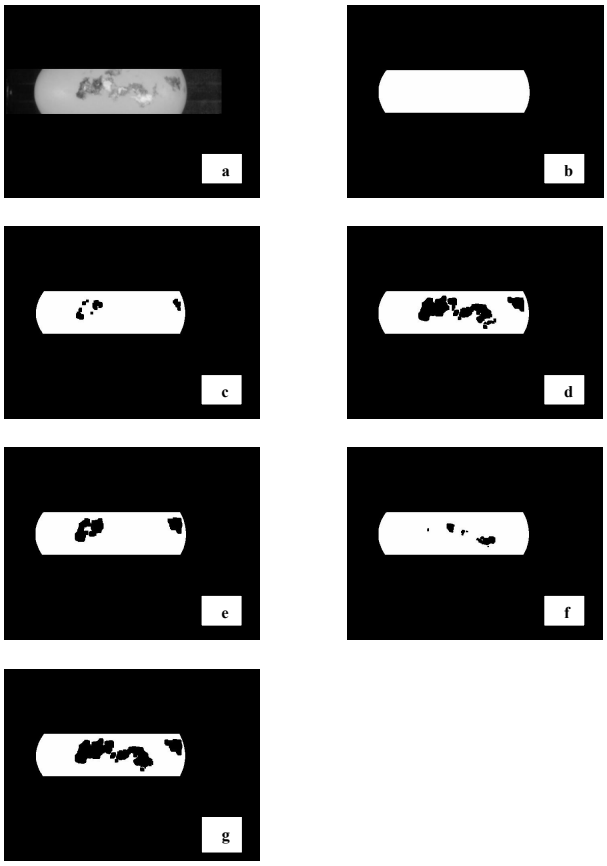

Figure 2: (a): original image; (b): result of section 1; (c): result of detecting dark stains part 1; (d): result of detecting dark stains part 2; (e): result of detecting dark stains part 3; (f): result of detecting white stains; (g): result of adding all images with stains.

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DEVELOPMENT OF A METHODOLOGY FOR CALCULATION OF YOUNG'S MODULUS OF EGGSHELL USING VIBRATION MEASUREMENTS.

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Keywords : Young's Modulus, Eggshell, Vibration, Modale analysis

Abstract

The elastic-modulus of the eggshell is a measure of its material strength independent of its shape. A new technique for the measurement of the dynamic E-modulus of an eggshell is described and evaluated. This technique is based on the vibrational behaviour of a clamped rectangular beam. Modal analysis of a curved shell segment made it possible to transform the formula for the E-modulus of a rectangular beam into the formula for a curved segment. A test set-up was developed, to enable the measurement of the resonant frequency of a curved shell segment. Twelve Hisex® eggs were measured to validate the technique. The average E-moduli of the eggs was $2{,}897 * 10^{10}$ N/m². The variance between the E-modulus of the eggs was $2.536 * 10^{9}$ N/m². These values correspond with the values mentioned by other investigators. Furthermore, the variance between measurements on the same shell segment was $4.97 * 10^{7}$ N/m² proving that the test set-up provides repeatable measurements.

Résumé

Le module d'élasticité de la coquille est une mesure de la solidité de ce matériau indépendant de sa forme. Une nouvelle technique de mesure dynamique de l'E-module d'une coquille d'oeuf est décrite et évaluée. Cette technique est basée sur le comportement vibratoire d'un faisceau rectangulaire fixé. L'analyse modale d'un segment incurvé de coquille permet de transformer la formule de l'E-module pour un faisceau rectangulaire en une formule pour un segment incurvé. Un dispositif d'essai a été développé pour permettre la mesure de la fréquence de résonance d'un segment

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incurvé de coquille. Douze oeufs de poules Hisex ont été utilisés pour valider la technique. La moyenne des valeurs de E-modules des oeufs étaient de 2.897 * 10^{10} N/m². La variabilité entre les valeurs de E-module des oeufs était de 2,536 * 10^{9} N/m². Ces valeurs correspondent aux valeurs mentionnées par d'autres équipes. En outre, la variabilité entre les mesures sur un même segment de coquille était 4,97 * 10^{7} N/m² montrent que le dispositif d'essai fournit des mesures répétables.

Introduction

Several methods exist to determine the modulus of elasticity of a material. Some determine the static modulus of elasticity, E_s, from the stress-deformation curve. Others measure the dynamic E-modulus, E_d, by sonic or ultrasonic testing. In the case of sonic testing, the dynamic modulus of elasticity can be derived from the resonant frequency of a flexural vibration. A large variation for elastic modulus values of eggshells is to be found in the literature. All values quoted in table 1 result from static measurements. Following the simulations done by Bain (1990) a default E-modulus can be set at $3*10^{10}$ N/m².
Other parameters describing eggshell material properties and referred to in the literature are the Poisson ratio and eggshell density. The value of the Poisson ratio frequently used for the eggshell is 0,307 (Manceau and Henderson, 1970). The density of the eggshell material is 2400 kg/m³, which equals the material density of the calcite crystal in the palisade layer (Carter, 1968).

Table 1 Overview of different values of E-modulus of eggshell material

Author	Test method	E-modulus (x 10^9 N/m²)
Bain (1990)	Non destructive distributed external load	26,3 – 34,1
Rehkugler (1963)	Compressed circular ring	10,5 – 20,7
Tung et al. (1969)	Compressed whole eggs	45,7 – 46,9
Manceau and Henderson (1970)	Distributed external load	17,7

Materials and Methods

1 Calculation of E-modulus based on the vibration of a curved shell segment

1.1. Calculation of Young's modulus for a flat plate

The resonant frequency for a flat plate clamped at one side and free at the other sides is given in Equation 1 (Blevins 1993). It can be used as a first approximation for the modus of elasticity of the eggshell.

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$$f_{flat\ plate} = \frac{\lambda^2}{2 \cdot \pi \cdot a^2}\left[\frac{E \cdot h^3}{12 \cdot \gamma \cdot (1-\upsilon^2)}\right]^{\frac{1}{2}} \qquad (1)$$

with f = resonant frequency [Hz] υ = Poisson's ratio [-]
E = modulus of elasticity [N/m²] h = shell thickness [m]
a = projected lateral shell length [m] γ = mass per unit area [kg/m²]
λ^2 = coefficient dependent on vibration mode

1.2. Modal analysis of a curved shell segment

A modal analysis of the shell segment, clamped at one end, was done with the software packet Pro/Mechanica 2000i[2]. Modelling was done for two reasons, first one needs to know which vibration mode is measured in this test set-up since the coefficient λ depends on this vibration mode. Second, by modelling a curved shell segment a correction factor for the shell curvature can be obtained.

The shell segment constructed in PRO/Mechanica had average dimensions given by a thickness of 350 μm, 26 mm long, 10 mm wide and a radius of curvature being equal to 20 mm. The shell length in the model corresponds to the free length of the shell segment. The boundary condition that was imposed in PRO/Mechanica was that the segment was clamped at one small side. Material properties assigned to the shell were: $E = 3*10^{10}$ N/m², $\nu = 0.307$, $\mu = 2400$ kg/m³. All vibration modes with a frequency below 3000 Hz were calculated in PRO/Mechanica for the described shell segment. Three vibration modes were found. The first mode occurs around 300 Hz and is the first flexural mode. The second mode, around *900* Hz, is a more complex one. Visualization in PRO/Mechanica revealed that the shell segments in this vibration mode primarily move in a plane perpendicular to the first mode. The third mode occurs around 1600 Hz and is the second purely flexural mode. Figure 1 visualizes the pure flexural modes.

Figure 1. Visualization of the flexural modes: the first (left) and the third (right) vibration mode as simulated in PRO/Mechanica. The position of the shell segment in rest is depicted together with the utmost position during the vibration of the segment.

1.3. Correction factor for shell curvature.

The influence of the radius of curvature and of the E-modulus on the resonant frequency of the third vibration mode was investigated. The resonant frequency of the

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third vibration mode is investigated because the developed test set-up (cfr. infra) measures the third vibration mode of the shell segment: 1) the resonant frequencies measured in this test set-up approximate the value of 1600 Hz, which was the resonant frequency of the third vibration mode as calculated in PRO/Mechanica 2). Measurements of the amplitude of the vibration on several points of the shell segment revealed the same vibration pattern as obtained in PRO/Mechanica. Therefore the resonant frequency of the third vibration mode was used to calculate the elastic modulus. To investigate the effect of the radius of curvature on the vibration of the shell segment, the resonant frequency -of the segment with properties as mentioned above- was calculated in PRO/Mechanica for different radii and E-moduli, whilst all other parameters were kept constant. The radii and E-moduli used in the calculations were respectively 19, 20, 21, 22, 23 mm and $1*10^{10}$, $2*10^{10}$, $3*10^{10}$, $4*10^{10}$ and $5*10^{10}$ N/m². Furthermore, the resonant frequency was also calculated for a flat plate with the same dimensions.

A ratio of resonant frequencies (resonant frequency of curved shell/resonant frequency of flat plate) was calculated. Both resonant frequencies were simulated in PRO/Mechanica for a plate with the same dimensions and the same E-modulus as the curved shell. Different E-moduli were used during the calculations to check whether the influence of the radius of curvature depends on the value of the E-modulus. The influence of both E-modulus and the radius of curvature on the ratio was investigated by means of a regression analysis. To obtain a normal distribution of the dependent variable, needed for a correct analysis, the ratio of resonant frequencies was transformed using the arcsine transformation (Neter et al., 1996). Regression analysis on the transformed variable revealed no significant effect of the E-modulus on the ratio of resonant frequencies ($p = 0.9857$). The radius of curvature on the other hand was very significant ($p < 0.001$). Thus a correction factor for the shell curvature needs to be taken into account and the correction factor accounts for all E-moduli.

To obtain the correction factor the ratio of resonant frequencies is plotted against the inverse of the radius (figure 2) and a trendline is fitted. The inverse radius is plotted instead of the radius of curvature because the latter is infinite for a flat plate.

$$\frac{f_{curved\ plate}}{f_{flat\ plate}} = -30.22 \cdot 10^{-6} \cdot (\frac{1}{R})^2 - 1{,}022 \cdot 10^{-3} \cdot (\frac{1}{R}) + 1 \qquad (2)$$

with $f_{curved\ plate}$ = resonant frequency curved plate [Hz]
$f_{flat\ plate}$ = resonant frequency flat plate [Hz]
R = radius of curvature at the equator [m]

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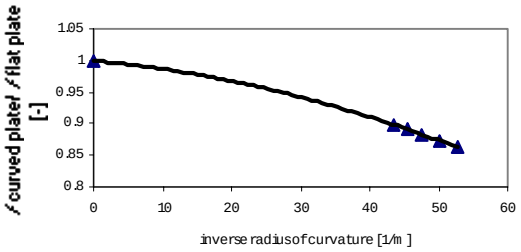

Figure 2. Graphical presentation of the ratio of resonant frequencies against the inverse radius

1.4. Calculation of the E-modulus for curved shell segment

Substituting Equation 2 into Equation 1 makes it possible to calculate the E-modulus of a curved shell segment on the basis of a resonant frequency measurement

$$E = \frac{48 \cdot \pi^2 \cdot f^2_{curved\ plate} \cdot a^4 \cdot \gamma \cdot (1-\nu^2)}{\lambda^4 \cdot h^3 \cdot (-30.22 \cdot 10^{-6} \cdot (\frac{1}{R})^2 - 1.022 \cdot 10^{-3} (\frac{1}{R}) + 1)^2} \qquad (3)$$

The parameter that remains unknown is the coefficient λ, which depends on the vibration mode. Because the test set-up measures the third vibration mode its value can readily be obtained from Blevins (1993) and equals 4,643. This is the value for a flat plate with the same dimensions as the shell segment, clamped at one side and free at the other sides.

2. Development of a test set-up, to measure the resonant frequency of curved shell segments

2.1. Preparation of test specimen

Three rectangular sections, dimensions 10 mm by 35 mm, were marked along the equator of each egg. The rectangles were sawn out of the shell. Thereafter the membrane was manually removed from the shell and the dimensions of each shell segment were measured; i.e. projected length, width and thickness. The measured length was transformed to an arc length according to:

$$arc\ length = 2 \cdot \sin\left(\frac{projected\ length}{2 \cdot \pi \cdot R}\right) \cdot R \qquad (4)$$

where R denotes the eggshell radius at the equator.

2.2. Test set-up

A paperc holding the shell segment was clamped in a vice. Underneath the shell segment, a loudspeaker was installed. A burst chirp with frequency range of 0 to 3000 Hz was used to excite the shell segment. Above the shell segment a laser vibrometer was mounted to capture the vibration response of the test specimen. The laser beam of the vibrometer is positioned at the free, small end of the shell segment

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2.3. Determination of the vibration mode measured by test set-up

When a shell segment is excited by this test set-up, a small peak is detected in the power spectrum around 300 Hz, which corresponds to the frequency of the first vibration mode as detected in PRO/Mechanica. However, this resonant frequency was not used in the calculations of Young's modulus because it is weakly excited in this test set-up. Figure 3 shows the recorded time signal from the vibration of an eggshell and its corresponding frequency signal. The second mode detected in PRO/Mechanica, around *900* Hz, was not visible in the power spectra. Because the third mode was dominantly excited in this test set-up, this mode was used to calculate the E-modulus in this work.

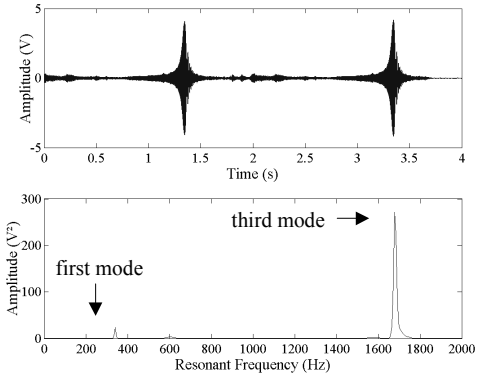

Figure 3. Recorded time signal (top) together with the power spectrum (bottom) of the vibrating eggshell segment.

Results and discussion

In this work 12 eggs from the genetic layer Hisex® were measured. At the time of measurement the birds were 66 weeks old. A total of 36 shell segments were measured originating from the 12 eggs. To check whether the measurements of the E-modulus are repeatable, each segment was measured three times. The average E-modulus of the 12 eggs, obtained with the proposed technique, was $2{,}897 * 10^{10}$ N/m². The variance between the E-modulus of the eggs was $2.536 * 10^{9}$ N/m², These values are in line with the values for the E-modulus mentioned by other investigators. Furthermore, the variance between measurements on the same shell segment was $4.97 * 10^{7}$ N/m² proving that the test set-up provides repeatable measurements.

In this work the elastic modulus of eggshells was obtained from dynamic measurements. The results show that the value of the dynamic or the static E-modulus of an egg shell coincide well. The technique described in this work makes it possible

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to measure the E-modulus several times on the same egg. Moreover, each sample can be measured several times, with a high repeatability.

Acknowledgments

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CONTENTS

APPLICATION OF LOW RESOLUTION ^{1}H NMR SPECTROSCOPY (LR ^{1}H MR) FOR DETERMINING INTERNAL EGG QUALITY – CORRELATIONS BETWEEN RELAXATION TIME T2(2) AND INTERNAL EGG QUALITY PARAMETERS

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Keywords : Egg quality, LR ^{1}H NMR, relaxation time, egg proteins

Abstract

Eggs from caged hens were collected at defined hens` age during the laying cycle and investigated under varying storage conditions applying LR ^{1}H NMR on intact eggs as well as physical and chemical methods on egg contents. Using a statistical model based on polynomial regression it can be shown that there is a dependency of the T2(2) relaxation time upon storage time as well as the laying age of the hens. In contrast, T2(1) depends only upon laying age of the hens and does not change significantly during storage. R^2 values between relaxation time T2(2) and Haugh units were in the range of 0,57 – 0.98 for the various storage regimes applied. There were no significant correlations between T2(2) values and changes in the content of VMOI, VMOII, lysozyme and conalbumin.

Résumé

Des oeufs de poules en cages ont été prélevés à différents âges au cours d'une année de ponte et ont été conservés dans différentes conditions avant que leur contenu soit analysés par 1H RMN à basse résolution ou par les méthodes chimiques standard. Il a été démontré, en utilisant un modèle statistique de régression polynomiale, que le temps de relaxation, T2(2) est dépendant du temps de stockage mais qu'il est également influencé par l'âge des poules. Par contre la composante T2(1) de la relaxation n'est influencée que par l'âge des poules, non par la durée de conservation des œufs. La corrélation (R2) entre le temps de relaxation T2(2) et l'estimation de la qualité du blanc par les unités Haugh est très élevée dans les différentes conditions

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testées. Il n'y a par contre pas de corrélation entre les valeurs de T2(2) et les modifications des teneurs de protéines de l'œuf : VMOI, VMOII (protéines de la membrane vitelline), lysozyme et ovotransferrine.

Introduction

There exist various physical and chemical methods for the determination of egg quality:
1) The height of the air space, which increases during storage, is used as a measure of the freshness of eggs. It is the result of diffusion of carbon dioxide and evaporation of water via the egg shell. However, these values are not very reliable as the initial height varies from egg to egg in association with the laying period of the hen and the prevailing environmental conditions (Pingel, 1993).
2) The pH of the egg white is another parameter which changes after laying. It rises from values of about 7,5 to 9,6 during storage and is dependent upon time and temperature. However, these values vary considerably and it is necessary to destroy the eggs to obtain these values.
3) During storage the viscosity of the egg white and the stability of the vitelline membrane decreases. These characteristics are applied in different procedures for the determination of the age of eggs, e.g. albumen index (Heiman and Carver, 1936) and Haugh unit (Haugh, 1937).
There is also a variety of chemical changes in egg components which can be used as indicators of egg quality. The increase in pH correlates with the liquefaction of the egg white and the decrease in viscosity (Heath, 1977). Aggregated ovomucin is dissociating into α-ovomucin and β-ovomucin in the course of storage (Miller et al., 1982). Ovalbumin is transformed into S-ovalbumin (Smith and Back, 1962).
Most of the above mentioned methods applied for the determination of egg quality require the destruction of eggs. An alternative fast, non invasive and non destructive method for the determination of internal egg quality is low resolution ^{1}H Nuclear Magnetic Resonance (LR ^{1}H NMR) spectroscopy (Capozzi et al., 1999).
Components of the egg white but also of the vitelline membrane deteriorate during storage. These changes in the egg caused either by physico-chemical mechanisms or microbial growth leading to alterations in the inner egg quality can be detected by means of LR ^{1}H NMR and compared to results obtained by Haugh unit measurement and HPLC analysis.

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Materials and Methods

Standardised eggs from caged Lohmann Brown hens were collected at five defined times during the laying cycle ($26^{th}/31^{st}/38^{th}/46^{th}/63^{rd}$ week) and stored under conditions varying in combination of temperature (5/20/25 °C), atmosphere (CO_2/air) and lighting (dark/artificial light) at a constant relative humidity, r.h. of (60 %). Collectives of up to 1000 eggs were stored for 5 weeks maximum and used for investigations performed by means of LR ^{1}H NMR spectroscopy, Haugh units determination and HPLC analysis.

Applying the LR ^{1}H NMR spectrometer (mq 10 NMR Analyzer, Bruker, Karlsruhe, Germany) the transversal (spin-spin) relaxation times T2(1) and T2(2) of intact eggs were determined at an excitation frequency of 7,5 MHz. Scanning time was 20 sec consisting of four scans. This method implies the insertion of individual eggs into a homogeneous magnetic field using appropriate teflon cuvettes (diameter 46 mm). The hydrogen nuclei in the egg are excited by radio frequency pulses. The resulting relaxation times T2(1) and T2(2) reflect the times required for the excited system to return into its relaxed state.

Haugh units were determined by a special Haugh unit meter (TSS-Technical Services and Supplies, York, England). The instrument allows the determination of the egg weight and after breaking the egg the height of the thick egg albumen. Haugh units are automatically calculated.

The egg white proteins lysozyme and conalbumin as well as the vitelline membrane outer proteins VMOI and VMOII were separated by means of HPLC (Beckman System Gold, Munich, Germany) on a Poroshel 300SB-C18 column (Agilent Technologies, Karlsruhe, Germany) and their content estimated via the peak areas.

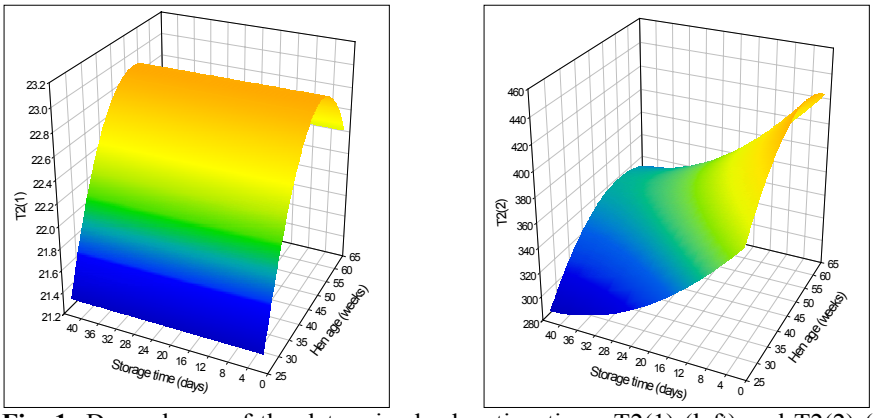

Fig. 1: Dependency of the determined relaxation times T2(1) (left) and T2(2) (right) [in ms] upon the storage time and the laying age of the hens. Storage conditions: 20 °C; 60 % r.h.; air/darkness; 350 eggs were used in total.**Results and discussion**

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According to the results of a statistical model applying polynomial regression T2(1) correlates to the laying age of the hens (Fig. 1, left), whereas there is no change of T2(1) in dependency upon the storage time. This means, that there are no significant changes with respect to T2(1) during storage of the different egg collectives collected at defined times ($26^{th}/31^{st}/38^{th}/46^{th}/63^{rd}$ week) during the laying cycle of caged hens. On the other hand there is a tendency, that the T2(1) values of the eggs are increasing in the middle of the laying cycle of the hens, whereas at the end of the laying cycle the T2(1) values are decreasing again.
Regarding T2(2) values in dependency upon storage time (Fig. 1, right) there can clearly be shown a decline of the relaxation time of the various egg collectives collected at defined times ($26^{th}/31^{st}/38^{th}/46^{th}/63^{rd}$ week) during the laying cycle in the course of storage at 20 °C, 60 % r.h. under air and darkness. A dependency of the T2(2) values upon the age of the hens during the laying cycle can also be observed. The course of variation is in a similar way expressed as in the case of T2(1). However, alterations concerning T2(2) are much more significantly expressed as compared to T2(1).

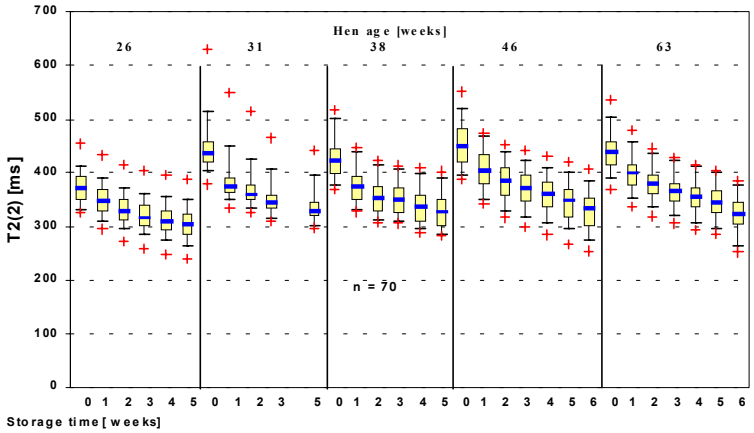

Fig 2: Course of T2(2) values determined for egg collectives collected at defined times ($26^{th}/31^{st}/38^{th}/46^{th}/63^{rd}$ week) in the laying cycle of caged hens during storage at 20 °C, 60 % r.h. under air in darkness.

In figure 2 the course of T2(2) values during storage at 20 °C, 60 % r.h. under air in darkness for egg collectives collected at defined times ($26^{th}/31^{st}/38^{th}/46^{th}/63^{rd}$ week) during the laying cycle is shown in form of Box-Whisker-Blots. The dependency of

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the T2(2) values upon storage time and laying age of the hens can clearly be recognized as already demonstrated in figure 1.

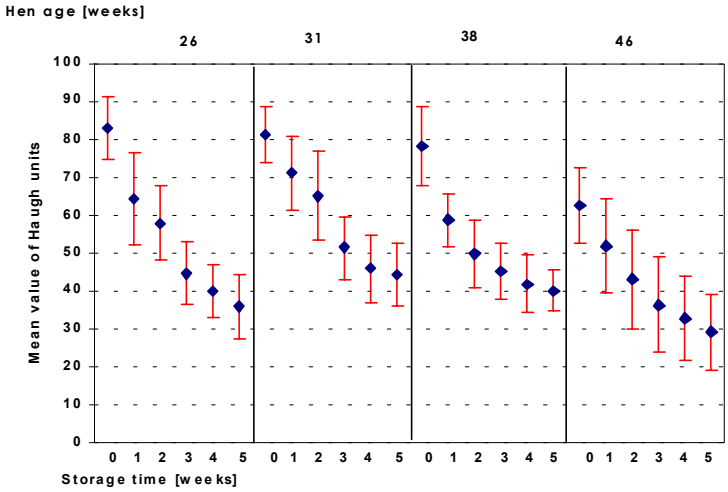

Fig 3: Course of mean values of Haugh units determined for egg collectives stored at 20 °C, 60 % r.h. under air in darkness collected at defined times ($26^{th}/31^{st}/38^{th}/46^{th}$ week) during the laying cycle.

In figure 3 the course of mean values of Haugh units determined for egg collectives stored at 20 °C, 60 % r.h. under air in darkness collected at defined times ($26^{th}/31^{st}/38^{th}/46^{th}$ week) during the laying cycle is depicted. Egg collectives of the 46^{th} week of caged hens show significantly lower Haugh units than eggs collected in an earlier stage reflecting a lower viscosity of the egg albumen. At this time in the laying cycle of hens the eggs are normally larger than in the beginning. This means, that at a constant protein content the eggs contain a higher water percentage resulting in a lowered viscosity.

R^2 between the relaxation time T2(2) and the Haugh units determined for eggs collected in the 26^{th} week of the laying cycle of caged hens revealed to be for storage at 20 °C and 60 % r.h. under a) carbon dioxide 0,86 b) air/darkness 0,84 and c) air/artificial light 0,98, whereas the R^2 values between T2(2) and the resulting mean Haugh units were with respect to the different storage temperatures: 0,57 (5 °C); 0,85 (20 °C) and 0,88 (25 °C). Only at higher temperatures a more narrow correlation between these two parameters was observed. Changes in eggs like liquefaction and increase of pH are influencing T2(2) as well as the resulting Haugh units.

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The content of lysozyme in the egg white as well as in the vitelline membrane is not significantly changing during storage up to 36 days at elevated temperatures of 20 °C and 60 % r.h.. In contrast there is a declining tendency regarding the mean values of the vitelline membrane outer proteins VMOI and VMOII during storage. In the case of VMOII there is a significant decrease of the content especially during the first two days after laying. There was no obvious correlation between T2(2) and the changes in the content of lysozyme and conalbumin in the egg white.
In summary, LR ^{1}H NMR spectroscopy is capable of detecting storage dependent changes in eggs. Because of the variation from egg to egg, it cannot be used as an absolute freshness indicator for individual eggs. However, the method can be applied for this purpose, if relaxation times of larger egg collectives are taken into consideration.

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NMRD STUDY OF HEN EGG ALBUMEN

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Keywords : NMRD, Relaxometry, Albumen thinning.

Abstract

Thick albumen from 10 oiled and 10 not oiled eggs laid by Isa Brown Warren hens (aged 20 weeks) were analyzed for longitudinal relaxation time at variable magnetic field (NMRD) both immediately and one week after laying. The average $<\tau_c>$, β and α parameters extracted from the dispersion profiles agree with the hypothesis of an increased interaction among the albumen proteins. A second unrelated process might be present in which some of the albumen proteins undergo purely structural changes.

Résumé

L'albumen épais provenant de 10 oeufs huilés et 10 autres non huilés produits par des poules Isa Brown Warren (âgées de 20 semaines) ont été analysés par la mesure du temps de relaxation longitudinal à des champs magnétiques variables (NMRD) immédiatement et une semaine après la ponte. La moyenne des paramètres $<\tau_c>$, β et α extraits de la courbe de dispersion NMRD est en accord avec l'hypothèse d'une augmentation de l'interaction entre les protéines de l'albumen. Un second processus non-relationnel peut être observé dans lequel une partie des protéines de l'albumen sont sujets à des changements purement structurels.
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Introduction

Egg quality is assessed by a number of well established methods, each exploiting some naturally occurring phenomena in eggs after laying. The quality of the egg albumen is usually determined through the measure of the so-called Haugh Index (HI), which is related to the firmness of the albumen and also gives an indication of the egg's age.

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Although the significance of the HI as an ageing indicator has sometimes been questioned (Silverside and Villeneuve, 1994), its use for the determination of the interior quality of eggs is widespread.

The mechanism by which albumen undergoes thinning is still not completely understood, nor is there a general agreement in the literature concerning the few hypotheses already put forward. In general, it is agreed that the high molecular weight glycoprotein ovomucin is responsible for the semi-solid appearance of the thick white through chain entanglement and/or electrostatic interactions with lysozyme and metal cations (Robinson, 1987). The fact that the amount of albumen ovomucin progressively decreases during ageing is taken as proof of its importance in the thinning process (Robinson and Monsey, 1972a,b; Kato et al., 1979). One of the suggested explanations of albumen thinning implies an enhancement of the complexation between ovomucin and lysozyme as pH increases during ageing. The complex would then precipitate causing ovomucin depletion (Robinson and Monsey, 1972b; Miller at al., 1982). In another explanation, the natural increase of the egg's pH during ageing is thought sufficient to bringing about glycosidic bond cleavage between -OH containing ovomucin aminoacids (serine and threonine) and its negatively charged (at egg's pH) polysaccharide moieties (Kato et al., 1979). This would gradually prevent ovomucin from interacting sufficiently with lysozyme, thus making the three dimensional structure of the albumen collapse. The destruction of some type of physical or electrostatic protein lattice also agrees with the notion that albumen changes from a non-Newtonian to a more Newtonian fluid as thinning proceeds (Robinson and Monsey, 1972a).

Although in the above explanations the presence of water is overlooked, it must not be forgotten that water makes up 88% of the average albumen weight and that (at the egg's pH) the water protons are in fast chemical exchange with the labile proteins' protons (Halle and Denisov, 2001). The dynamic of the water is thus influenced by the presence of the proteins. More importantly, the modifications that take place in the albumen proteins may be sensed by the interacting water, provided the time scale of this interaction is not “too fast” (Halle and Denisov, 2001). In this case, nuclear magnetic resonance dispersion (NMRD) experiments can be of valuable help in detecting the type of change the albumen proteins have undergone.

This work presents for the first time an NMRD study of the thick albumen obtained from fresh and one-week aged eggs. As it is well known (Romanoff and Romanoff, 1949) that thinning is greatly reduced if the egg's pH is kept at the value it has at laying (about 7.6), half of the eggs used in these experiments were oiled with paraffin oil so as to provide a pH-independent reference for the measured effects.

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Materials and Methods

Eggs. Thirty eggs from Isa Brown Warren hens of 20 weeks of age were collected by hand from a local farm while they were still warm. Half of the eggs were immediately oiled by dipping them in paraffin oil (Carlo Erba, Italy). Each oiled egg was further wrapped in polyethylene film to further avoid CO_2 loss from the shell. Five oiled and 5 non oiled egg were taken to the laboratory and analyzed by NMRD approximately 6 hours after laying. The remaining eggs were stored at 20 degrees and analyzed one week later.

Traditional analyses. The eggs were analyzed for albumen pH, Haugh index (HI) and yolk index (YI). Each egg was carefully opened and the content laid on a flat glass surface placed on a metal stand with adjustable legs for levelling. Albumen height was measured with a digital micrometer following the USA Department of Agriculture guidelines (Anonymous, 2000). The value of the pH was measured with a Crison 507 pH meter equipped with a Double Bore electrode (Hamilton) which was inserted directly into the thick albumen. Yolk index was obtained as the ratio between yolk height and width (Romanoff and Romanoff, 1949).

NMRD. Nuclear magnetic resonance dispersion profiles were recorded with a Stelar fast field cycling Spinmaster relaxometer. For each sample sixteen longitudinal relaxation rates (R_1's) were obtained at logarithmically equispaced magnetic fields in the range 10 Khz - 20 Mhz. Total measuring time was about 30 minutes per egg.

Data analysis. The NMRD curves were analyzed according to the model-free approach by Halle et al. (1998). This method was specifically studied for obtaining meaningful NMRD parameters even when the relaxation data in a plot of R_1 vs the logarithm of the Larmor frequency appear "stretched". Recently, a robust experimental study has confirmed the validity of the model-free approach (Bertini et al., 2000). Each NMRD curved was fitted to the following equation:

$$R_1 = \alpha + \beta\left[0.2J(\omega) + 0.8J(2\omega)\right] \qquad (1)$$

where $J(\omega)$ is defined as a sum of Lorenztian functions,

$$J(\omega) = \frac{\sum_{n=1}^{N} c_n \frac{\tau_n}{1+(\omega\tau_n)^2}}{\sum_{n=1}^{N} c_n} \qquad (2)$$

The choice of N in Eq. 2 was based on the F-statistics, as suggested by Halle et al. (1998). It was pointed out by Halle et al. (1998) that the τ_n's obtained from the fit have limited individual significance while their c-weighted average ($<\tau_c>$) is expected to be phenomenologically meaningful. All calculations presented in this work were carried out with the open-source program "R" (http://www.r-project.org) by Ihaka and Gentleman (1996).

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Results and Discussion

The results of the traditional analysis methods for the present eggs are given in Tab. 1.

	Oiled (fresh)	Oiled (aged)	Not oiled (fresh)	Not oiled (aged)
pH	7.77^{a}	7.79^{a}	8.50^{b}	9.10^{c}
HI	77.7^{d}	83.8^{d}	68.5^{e}	56.7^{f}
YI	0.44^{g}	0.44^{g}	0.42^{g}	0.42^{g}

Table 1 (different letters indicate significant differences with $p<0.05$).

The NMRD experimental points for thick albumen samples obtained from 10 oiled and 10 non oiled eggs are shown in Fig. 1A and B, respectively, together with the best fit curves calculated with Eq. 1. It appears that the R_1's of the fresh albumen samples are always lower than the R_1's of one-week aged albumen at all Larmor frequencies, and that the R_1's of the albumen samples from non oiled eggs are, on average, always higher than those from oiled eggs.

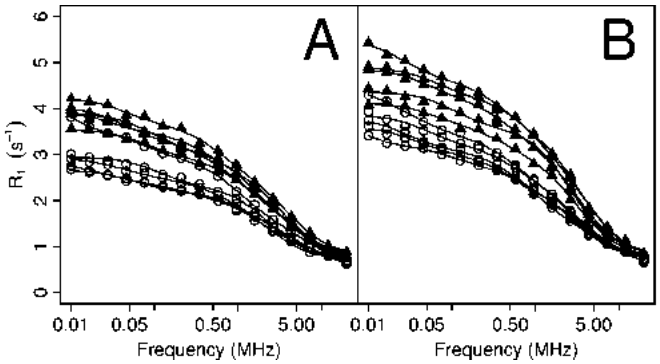

Fig. 1 (circles: fresh albumen samples; triangles: aged albumen samples)

One benefit of fitting "stretched" dispersion profiles with Eq. 1 is the possibility of separating the "static" (i.e. structural) and "dynamic" information hidden into the data points (Halle et al., 1998). Briefly, the shape of a dispersion profile may vary either because the macromolecules undergo aggregation (thus modifying their average hydrodynamic radius, hence $<\tau_c>$) or because of structural changes that modify the overall strength of the dipolar interaction (β) between water and proteins (such as, for example, folding/unfolding processes or loss of water molecules buried inside the proteins).

The separation between static and dynamic information is useful in the present case. In fact, it appears that $<\tau_c>$ decreases significantly ($p<0.05$) on passing from oiled to non oiled eggs, but does not change with the age of the eggs. (Fig. 2). The β value

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increases in one week by 30% in oiled eggs and by 20% in non oiled eggs; interestingly, if we take the β measured for fresh oiled eggs as the value one should obtain for thick albumen at laying, it turns out to have increased by more than 50% in 6 hours (i.e. pending analysis) for non oiled eggs. As expected (Capozzi et al., 2000), the α values (which are related to the presence of a relaxation rate high frequency plateau) tend to increase with albumen age (about 10% per week) and, similarly to what happens for β (and under the same assumptions), α increases more (about 30%) during the first 6 hours than during the week afterwards.

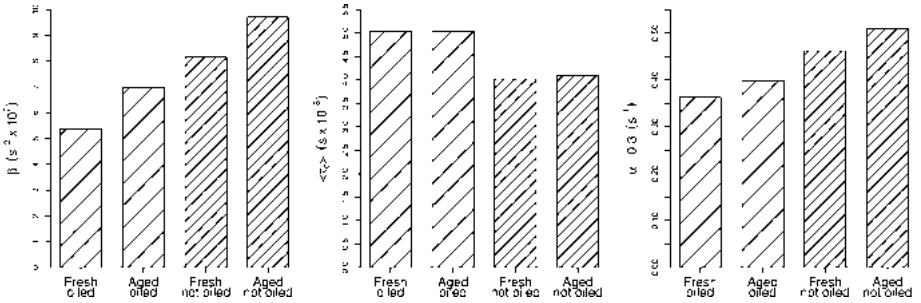

Fig. 2

It is tempting to speculate that the sudden increase of α and β in non oiled eggs is due to the structural modifications that take place within the albumen in the first hours after laying because of the pH increase (Tab. 1). The hypothesis of an increased interaction between ovomucin and lysozyme (Robinson and Monsey, 1972b; Miller at al., 1982) would fit nicely within the present results, as in this case the hydrodynamic radius of the new aggregate would become greater (hence lower $<\tau_c>$) and some water molecules would probably get trapped between the interacting macromolecules (thus causing an increase in β). However this view still does not explain why α and β increase with the age of the eggs; it is likely that a second unrelated process is active in which the albumen macromolecules undergo purely structural modifications during the time after laying.

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Session 9
Influence of production factors on egg quality

MARKER ASSISTED SELECTION TO IMPROVE EGGSHELL QUALITY: ESTABLISHING THE ASSOCIATION BETWEEN EGG PHENOTYPIC TRAITS AND 'CANDIDATE GENES'.

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Keywords : Selection, candidate gene, organic matrix, eggshell quality

Abstract

This paper describes the sampling strategy and eggshell quality (ESQ) assessment procedures which are currently being used to establish if an association exists between specific ESQ parameters and polymorphism in 'candidate' genes which encode for organic matrix proteins. Heritability estimates using half-sib analysis for some of the less documented ESQ parameters are also given.

Résumé

Cet article décrit les procédures d'évaluation de la qualité de la coquille (ESQ) et les stratégies d'échantillonnages qui seront couramment utilisées pour établir s'il existe une association entre les paramètres spécifiques de qualité de coquille (ESQ) et le polymorphisme des gènes candidats qui codent pour les protéines de la matrice organique de la coquille. L'héritabilité sera également estimée, en utilisant une analyse en demi-frères, pour certain des paramètres ESQ qui sont les moins bien documentés.

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Introduction

Breeding companies have been trying to improve the mechanical strength of eggshells by incorporating breaking strength, puncture strength, non-destructive deformation and specific gravity into their breeding programmes. Whilst most of these measures respond to selection, shell breakages in the field have not significantly decreased (Hamilton *et al*., 1979). According to De Ketelaere *et al.,* (2002) the dynamic stiffness (Kdyn) of an egg provides a global measure of eggshell strength. The forces applied to an egg in the field are also dynamic and so selection using a dynamic measurement could be of more benefit than a static measurement. However, heritability estimates and supporting field data are needed to justify these claims.

A different selection strategy, and one, which is becoming increasingly available, is to use genetic markers as tools to aid conventional breeding methods. Marker assisted selection (MAS) involves the selection of animals using DNA markers that will confer superior genetic merit for a specific phenotypic trait which is practically difficult to measure. This approach differs from conventional methods where the phenotypic trait itself is measured and used for selection. In terms of enhancing the mechanical properties of the eggshell, considerable progress has been made in the identification and characterization of the genes coding for individual eggshell matrix proteins, including those that have been implicated in directing crystal growth during eggshell formation (Gautron *et al.,* 2002). It is therefore possible that variation in the expression of one or more of these 'candidate genes' could account for measurable differences in an eggshells mechanical properties and hence its susceptibility to cracking and / or bacterial ingress.

The aim of this paper is to report on the progress of an ongoing investigation into the feasibility of using MAS and/or conventional genetic methods to improve the mechanical strength of eggs.

Materials and Methods:

The candidate genes considered in this study are Ovocleidin-116, Osteopontin, Ovocalyxin-32, Ovocalyxin-21, Ovotransferrin, Ovalbumin and Ovocalyxin-36. These genes were chosen as their products occur in the organic matrix of the eggshell and are likely to play a significant role in modulating and directing crystal growth during the shell forming process.

To establish if there was association between alternative alleles of any of these candidate genes and one or more phenotypic traits, it was necessary to have a resource population with DNA and pedigree information, and to develop the methodology to identify alleles at the candidate gene loci. A resource population from a Rhode Island Red pedigree line maintained by Lohmann Tierzucht was therefore created. The

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number of eggs from the offspring population, which could be assessed for eggshell phenotypic traits, was identified as a major limiting factor. A pilot study revealed that it would be advantageous to reduce the number of eggs sampled from each individual bird and to increase the number of animals sampled because of the high repeatability of eggshell traits. It is also advantageous to have large sire families to increase the chances of detection of association (Weller *et al*., 1990). Taking these considerations in to account resulted in a pedigree structure of 32 sires and 240 dams with an average of 64 offspring per half-sib family. Not all the sire families will be used in the association analysis; only the offspring of heterozygous sires. This reduces the amount of data, but will give marker effects estimated within families, and is less likely to be affected by background genetic differences in the population.

Blood samples for genomic DNA preparation were obtained from each sire, dam and offspring using EDTA as an anticoagulant. Genomic DNA was prepared using the GFX system (Amersham Pharmacia Biotech UK Ltd.,) adapted for avian blood. DNA is subject to mutation; many mutations are nucleotide substitutions that are known as single nucleotide polymorphisms (SNPs). These provide convenient markers to distinguish alleles of DNA and their inheritance. So far, polymorphism detection for Ovocleidin-116, Osteopontin and Ovocalyxin-32 has been carried out using direct sequencing of genomic DNA from 32 sires followed by comparison using Staden computer packages. In both cases, the DNA was amplified using primers designed to either published data or from genomic DNA sequences previously obtained utilising cDNA sequence information. SNP's were confirmed by restriction fragment length polymorphism or by AcycloPrime-FP SNP Detection Kits (PerkinElmer Life Sciences).

Phenotypic data was collated from the offspring population between 38 and 42 weeks of age by assessing 2 eggs per bird (n=2066). Initial measurements included egg weight (g), shape index (derived from measuring length/breadth in mm), dynamic stiffness (Kdyn) and damping ratio. Both the latter measurements were derived using the lab-scale test arrangement described by De Ketelaere *et al*., 2002. The breaking strength (F) at a compression speed of 20mm/min was subsequently derived. Each egg was emptied and the egg shells stored for assessment of the effective thickness by scanning electron microscopy, and the derivation of the elastic modulus and fracture toughness using the method and formulae developed by Bain 1990. Half-sib heritability estimates for the preliminary phenotypic traits including the dynamic stiffness (Kdyn) were estimated by REML using egg batch and hatch date as fixed effects and sire and dam as random effects.

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Results and discussion

The association analysis or the candidate gene approach assesses the effect of candidate genotypes on phenotypic traits, whilst allowing for additional variables (Dunn *et al.,* 2001). To assess a candidate gene loci for association, it is first necessary to detect a polymorphism which can be used to distinguish alleles at the specific gene loci. This approach has already yielded a number of significant associations between gene and trait in farm animals, crop plants and in human clinical trials (Spielmann *et al*., 1993; Rothschild and Soller, 1997).

The candidate genes in the current study were chosen on the basis that their products are known to occur in the organic matrix of the eggshell, which modulates and controls bio-mineralisation. Ovocleidin-116 is the most abundant organic matrix protein in the uterine fluid during the rapid phase of eggshell calcification and was the first novel eggshell matrix protein to be cloned (Hincke *et al*., 1999). The current study has revealed that polymorphism in Ovocleidin-116 exists in the experimental population, and this results in an alteration in the protein sequence. This may have implications for the flexibility of this protein. The frequency of the less common allele is 26%. Osteopontin is normally associated with bone but was identified in the eggshell by Pines *et al.,* 1995. Twenty-three polymorphic loci have been discovered for this gene including in the promoter region; these are all completely linked and represent two alleles. The frequency of the less common allele is 43%. Ovocalyxin-32 has only recently been identified and cloned (Gautron *et al*., 2002). This protein is predominately produced in the terminal phase of calcification and is localised in the outer part of the eggshell. Two polymorphisms have been confirmed for Ovocalyxin-32, one in an intron (frequency of less common allele 33%) and the second in the final exon, which alters the hydrophobicity of the protein (frequency of less common allele 9%). It is anticipated that similar polymorphisms in the genes coding for the other three organic matrix proteins will be detected.

The following phenotypic traits will form the basis of the association analysis with the organic matrix candidate gene alleles: egg weight, shape, dynamic stiffness, damping ratio, breaking strength, effective thickness, and elastic modulus and fracture toughness. According to Bain 1990, the latter two measures better reflect the modifying effects made by the organic matrix to the eggshells mechanical strength. If association analysis for these traits proves positive, genetic markers will become available for these specialised eggshell quality traits. These will then be available for introduction into selection protocols. Some of the other phenotypic traits are arguably more suited to conventional selection programmes. Heritability estimates for egg weight, shape, damping ratio, dynamic stiffness and shell breaking strength have therefore been presented in Table 1.

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Trait	Mean	SD	Heritability (SE)
Egg weight (g)	66.0	4.9	0.52 (0.15)
Shape Index	1.29	0.04	0.40 (0.12)
Damping Ratio (%)	3.00	0.53	0.26 (0.09)
Kdyn (kN/m)	1521	154	0.33 (0.11)
Breaking strength (N)	39.5	5.5	0.15 (0.07)

Table 1: *Mean, standard deviation and estimate of heritability using half-sib analysis ((sire variance *4)/sum of all the variance) for eggshell phenotypic traits.*

Egg weight is known to have a high heritability (Grunder *et al*., 1989) whilst breaking strength values are low in this study, possibly due to selection having been exerted on this trait. The moderate heritability estimate for Kdyn is of particular interest as it suggests that this trait could respond well to selection using conventional methods. The acoustic test is also non-destructive and easy to perform making it a more attractive option than current methodology. The relevance of this measure to an eggs' performance in the field however first needs to be confirmed. The damping ratio also has a moderate heritability, but it is difficult to see at this stage how this measure could be used since it is not clear if it is correlated to any functional trait.

In summary, genetic markers are intended as tools to aid selection and can be used instead of direct phenotypic measurements to maintain genetic progress for a specific trait. In terms of eggshell quality, the assessment of the effective thickness of the shell, and /or its elastic modulus and fracture toughness are more likely to be suited to MAS. MAS may also have a role in the selection for a wider range of traits in full sib males. In the population structures used in commercial breeding programmes sires have a large contribution to the next generation but no direct measurements can be made of their genetic merit for eggshell traits. MAS could therefore be valuable for the selection of full sib males for female traits.

Acknowledgments

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EGGSHELL QUALITY EVOLUTION DURING THE LAYING PERIOD WITH SPECIAL ATTENTION TO WITHIN AND BETWEEN HEN VARIABILITY

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Keywords : eggshell quality, mixed model, variability, evolution

Abstract

Three eggshell quality parameters (thickness, static and dynamic stiffness) were followed during the laying period of Bovans commercial layer hens. Each hen was housed in an individual cage, so that traceability of the eggs towards each individual hen was obtained. In order to be able to assess within hen and between hen variability, a linear mixed model was used. It was observed that the variability in terms of eggshell strength parameters between hens increased when hens became older. In addition, an increase in within-hen variability was observed. However, the average shell quality did not change drastically over the entire laying period. The increased incidence of cracked shells that is reported in practice is therefore related to a larger variability of the shell quality near the end of laying, and not so much to the fact that average shell quality decreases.

Résumé

Trois paramètres de qualité de la coquille d'oeufs (épaisseur, rigidité statique et dynamique) ont été suivis pendant la période de ponte de poules commerciales Bovans. Chaque poule était en cages individuelles, de manière à avoir un suivi individualisé de chaque œuf. Un modèle mixte linéaire a été employé pour évaluer la variabilité inter et intra poule. Nous avons observé que la variabilité entre poule de la solidité de la coquille d'oeuf augmentait avec l'age des poules. De plus une augmentation de la variabilité intra poule est aussi observée. Cependant en moyenne, la qualité de la coquille n'a pas fortement changé au cours de la période de ponte.

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L'augmentation du nombre d'œufs cassés qui est décrite est une conséquence d'une augmentation de la variabilité de la qualité de coquille à la fin de la période de ponte et pas tellement du fait de la réduction de la qualité globale de la coquille.

Introduction

Biological systems are characterised by their large natural variability and their time-dependent behaviour. This makes it difficult to ensure a high and consistent quality. In order to gain insight into such systems, not only is its average behaviour and how it evolves of interest, but even more the identification and quantification of the different sources of variability could prove to be very meaningful. Indeed, in cases where the variability is not constant over time, describing only the average trend could lead to wrong conclusions. An excellent example of such a biological production system is the laying hen. It is well known that eggshell quality changes during the commercial laying period (Wolford and Tanaka, 1970; De Ketelaere et al., 2002) with older hens producing, on average, weaker eggs in terms of some eggshell quality attributes such as static stiffness. However, this decrease is not seen for every shell quality parameter and is in some cases very low so that the increased incidence in broken eggs cannot always be explained by this average trend.
Our belief is that a concise analysis of the average trend and the evolution in variability of the studied quality attribute is a necessary step towards understanding and controlling the shell quality during the laying period. In order to achieve this, the theory of mixed models for longitudinal data offers a broad range of possibilities (Verbeke and Molenberghs, 2000) and will be discussed throughout this contribution.

Materials and Methods

In total 12 birds of the commercial layer Bovans brown were used throughout the analysis. All animals had hatched on the same day. At 17 weeks of age the chickens were collected and placed in traditional battery cages situated in a temperature and light controlled room. One bird per cage. The experiment started when the chickens were 33 weeks of age and continued till week 78. Every third week 4 eggs from each hen were collected. Eggs were first tested for cracks by means of the acoustic resonance frequency analysis (De Ketelaere et al., 2002). Eggs with faults in their shell were not used in this experiment. The same technique was used to measure the dynamic stiffness k_{dyn}. Static (k_{stat}) and dynamic stiffness and shell thickness were determined on each egg. Shell thickness was measured as the average of three

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equidistant points on the equator. Static stiffness was measured using a universal tensile and compression test machine (UTS Testsysteme GmBh., Ulm, Germany). Eggs were placed horizontally between two flat parallel steel plates and compressed at a speed of 1mm/min. The resolution of the sensor was 0.001 N. A maximum force of 10 N was exerted. Force and deformation were recorded throughout the test and used to calculate static stiffness defined as the slope of the force-deformation line. The measurement was repeated at three equidistant points on the equator of the egg.
The SAS software (SAS version 8.2, The SAS Institute Inc., Cary, NC, USA) was used throughout all statistical analyses. A longitudinal linear mixed model was used to analyse the data and is in its general form defined as (Laird and Ware, 1982):

$$\boldsymbol{Y_i} = X_i\boldsymbol{\beta} + Z_i\boldsymbol{b_i} + \boldsymbol{\varepsilon_i}\,, \qquad (1)$$

with $\boldsymbol{Y_i}$ the n_i–dimensional vector of all repeated quality measurements for the i–th hen; X_i the $(n_i \times p)$ design matrix of known covariates such as hen age; $\boldsymbol{\beta}$ a $(p \times 1)$ vector of fixed effects; Z_i a $(n_i \times q)$ matrix of known covariates (hen age) modelling how the quality attribute evolves over time for the i–th hen; $\boldsymbol{b_i}$ a $(q \times 1)$ vector of subject specific effects for which is assumed that $E(\boldsymbol{b_i}) = 0$ and $\boldsymbol{\varepsilon_i}$ the vector of residual components ε_{ij}, $j = 1,\ldots,n_i$. It is stressed that n_i denotes the number of repeated measurements for hen i, and not the number of hens. The random effects structure implies a covariance structure of a very specific form that allows estimating the contribution of within hen, between hen and measurement error variabilities (Verbeke and Molenberghs, 2000). For *fixed* effects model building purposes, maximum likelihood estimation (ML) was used rather than the default restricted maximum likelihood estimation (REML), which is the standard setting in the MIXED procedure of SAS. This allows nested models to be compared with a likelihood ratio test. To test whether *random* effects are needed in the model, the likelihood ratio test defined above was used but follows asymptotically a null distribution that is a mixture of chi–squared distributions (Verbeke and Molenberghs, 2000).In addition to the promising observation that the variance and its sub-contributions can be modelled, these repeated measures analysis require fewer experimental animals to elicit differences.

Results and discussion

Values for k_{stat} decreased linearly during ageing of hens, although the decrease was not significant ($P = 0.12$). The linear decrease in k_{stat} was also reported by De Ketelaere et al. (2002), although they found a significant decrease. The average value for the static stiffness was 148.1 kN/m at the beginning of the experiment, and decreased to 143.8 kN/m at the end (week 78), Figure 1.

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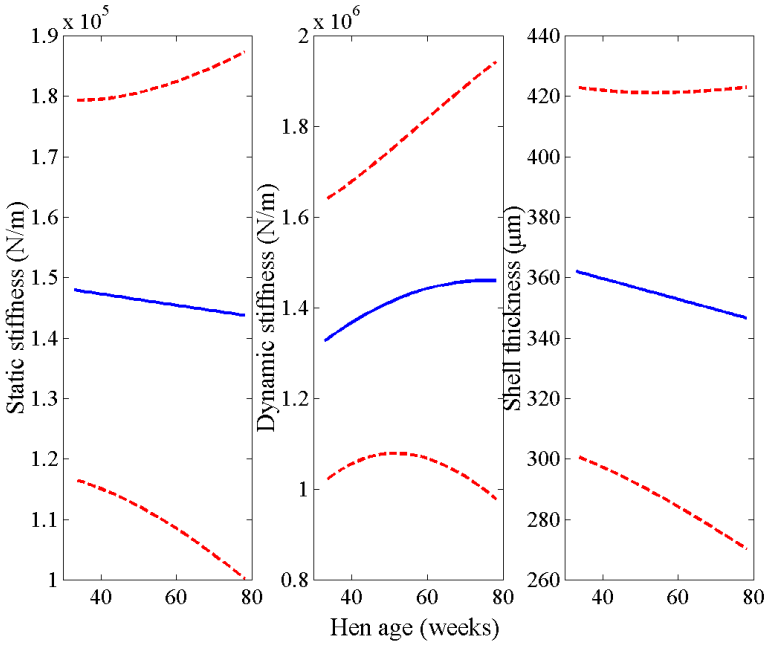

Figure 1: Evolution of the eggshell quality parameters during the laying period (full line) and 95 % confidence bands (dashed line). (a) static stiffness, (b) dynamic stiffness and (c) shell thickness.

The total data variability increased quadratically over the study from 2.5×10^8 to 5×10^8 $(N/m)^2$. A large part of the variability is due to the different static stiffness values of eggs belonging to different hens, and not so much due to within-hen variability (Figure 2 a). Although the influence of within hen variability is small compared to the between hen variability, a doubling in variability was also observed. In other words, even eggs originating from the same hen tend to be much more variable near the end of lay. The result of this increase in the variability in terms of k_{stat} is that at the end of lay, a significant increase in eggs that do not attain a certain 'threshold strength' can be observed. The threshold shown in Figure 2 b was arbitrarily chosen. Incorporating such a result in selection schemes may be useful since not only a high average quality is needed but also a consistent quality, as shown here. The analysis of the natural variability rather than the description of the average trend thus explains why under practical conditions more eggshell breakage is observed.

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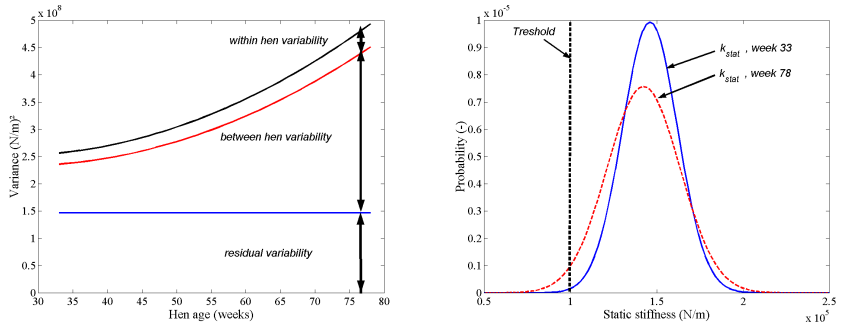

Figure 2: (a) Evolution of the variability in static stiffness during the laying period. (b) Distribution of static stiffness values at the beginning (full line) and at the end (dashed line) of lay.

For eggshell thickness, a similar behaviour was observed: a slight decrease in shell thickness was noticed being borderline significant ($P = 0.04$), Figure 1 c. On average, initial thickness was 363.4 µm and decreased to 349.0 µm at week 78. The increase in variance is not as pronounced as in the case of static stiffness (Figure 3 a), but causes in combination with the decreased average thickness a substantive increase in 'weak' eggs (Figure 3 b). Again, the between hen variability was found to be much larger than the within hen variability, and within hen variability increased from 90 µm² to 150 µm².

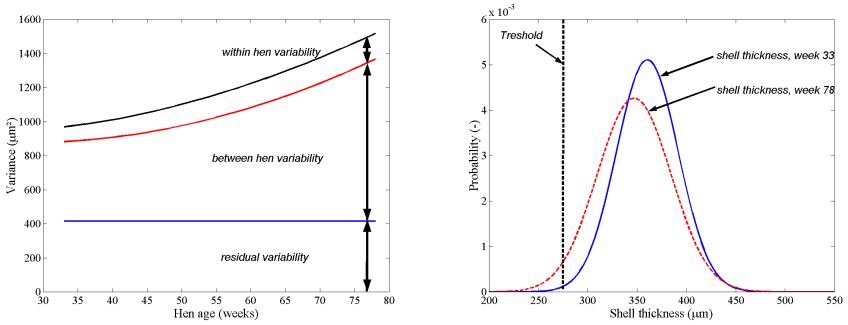

Figure 3: (a) Evolution of the variability in eggshell thickness during the laying period. (b) Distribution of eggshell thickness at the beginning (full line) and at the end (dashed line) of lay.

On average, the dynamic stiffness exhibits a contradictory pattern when compared to the static stiffness and shell thickness since its values increase with hen age from 1.32

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$\times 10^6$ to 1.46×10^6 N/m (P = 0.01), Figure 1 b. This is in line with the result reported by De Ketelaere et al. (2002) but seems, at first sight, contradictory with the increase in incidence of egg breakage near the end of lay as encountered in practice. However, as was the case for both previously mentioned quality attributes, there was also for the dynamic stiffness a significant increase in variability towards the end of the study (Figure 4 a). The effect of the within hen variability was almost negligible, caused by the fact that repeated measurements taken on the same egg produce the same values in terms of dynamic stiffness. This is due to the fact that the dynamic stiffness provides a global quality measure, and not a local one as shell thickness and static stiffness. The substantive increase in total variability causes a larger amount of 'weak eggs' near the end of lay, even given that, on average, the dynamic stiffness had increased. It has to be mentioned that the increase in incidence of weak eggs in terms of dynamic stiffness is not large, or could even be non-existent depending on the chosen threshold. In addition to these results, it must be stressed that eggs with identical strength in terms of one of the three quality attributes could be more likely to break at the end of lay when compared to the beginning of lay primarily because of egg weight ie increased egg weight gives rise to higher impact forces.

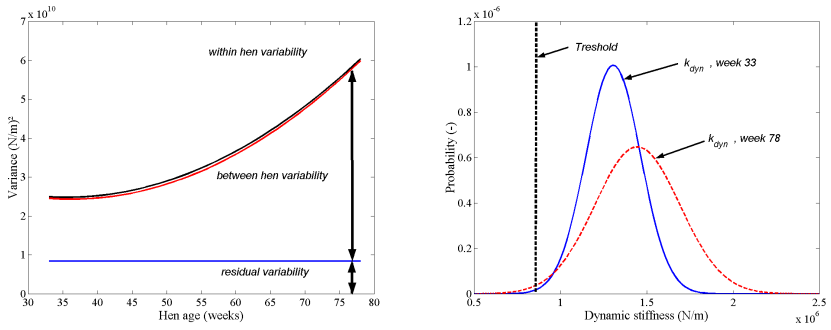

Figure 4: (a) Evolution of the variability in dynamic stiffness during the laying period. (b) Distribution of dynamic stiffness values at the beginning (full line) and at the end (dashed line) of lay.

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Conclusions

All three eggshell quality attributes (static and dynamic stiffness, eggshell thickness) provide the same information, namely that the incidence of weak eggs increases near the end of the laying period. This increased incidence is far more pronounced in the static stiffness and eggshell thickness values than in dynamic stiffness values. On average the dynamic stiffness of eggs increased during the laying period whilst the static stiffness and eggshell thickness decreased. In accordance to De Ketelaere et al. (2002) this fact again shows the relative independence of the dynamic stiffness when compared to static stiffness and eggshell thickness. This suggests that the dynamic stiffness may be a useful additional eggshell quality parameter to be included in selection schemes.

Acknowledgements

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GENETIC VARIABILITY OF THE ALBUMEN ANTI-MICROBIAL ACTIVITY OF HENS' EGGS

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Keywords: hen, egg-white, anti-microbial proteins, genetic, selection

Abstract

The bacteriological quality of table eggs could be reduced *via* an increase of broken/soiled eggs in the new laying hens' production systems (EU directive 1999/74). To optimise quality and security of table eggs laid in these systems, we propose to study the feasibility of selecting hens laying eggs with high anti-microbial properties. Indeed, several albumen proteins are involved in the inhibition of bacterial growth, especially lysozyme. We have first estimated, on 100 ISA Brown laying hens of different ages, the repeatability of the inhibition of the bacterial growth of *Salmonella* Enteritidis and *Staphylococcus aureus* by the albumen, as well as the albumen total proteins and lysozyme contents. Correlations were also estimated.

Résumé

La qualité bactériologique des œufs de table pourrait diminuer dans les nouveaux systèmes de production de poules pondeuses (Directive Européenne 1999/74). Afin d'optimiser la qualité et la sécurité des œufs de table pondus dans ces systèmes, nous étudions la faisabilité d'une sélection de poules pondant des œufs à fortes propriétés anti-bactériennes. En effet, plusieurs protéines de l'albumen possèdent de telles propriétés, dont le lysozyme. Nous avons dans un premier temps estimé la répétabilité de l'inhibition de la croissance bactérienne de *Salmonella* Enteritidis et *Staphylococcus aureus* par l'albumen, ainsi que la teneur de celui-ci en protéines totales et en lysozyme et ce, chez 100 poules pondeuses ISA Brown d'âges différents. Les corrélations ont également été estimées.

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Introduction

In 2012, conventional battery cages for laying hens will be replaced by furnished cages or alternative systems to improve hen's welfare (EU directive 1999/74). As a possible consequence, the number of cracked eggs or soiled eggs by hen's dejections or litter's dust could increase, as well as food-borne diseases. Thus, we are looking for ways to reinforce eggs' natural defences at the eggshell and the albumen levels. The present study is the first part of a project aiming at selecting layer-type hens with higher eggs' albumen anti-microbial properties. Indeed, numerous proteins confer such properties on albumen, especially lysozyme (Burley and Vadehra, 1989; Ibrahim et al., 2002). Our main objectives were to estimate the repeatability of the inhibition of *Salmonella* Enteritidis' and *Staphylococcus aureus*' growths (gram negative and gram positive bacteria, respectively) in albumen as well as that of albumen total proteins and lysozyme contents. A good repeatability is a prerequisite for the feasibility of a genetic selection because it corresponds to the superior limit of heritability. Those parameters have been measured in eggs laid by 100 ISA-Brown hens from a same pedigreed line, at two different laying periods: 22 to 25 and 35 to 38 weeks of age. Hen effect, age effect and correlations between all these parameters were also estimated.

Material and Methods

Eggs laid by 100 Isabrown hens from different families of a same pedigreed layer line were obtained from Hubbard Isa. They were collected at two given periods: the beginning and the middle of the laying cycle (22 to 25 and 35 to 38 week-old hens, respectively). For a given week, eggs from each hen were collected each day (Monday to Friday) and were stored at room temperature. For the analyses, eggs were rubbed with absorbent paper to clean their shells and to detect any cracks, then their surfaces were sterilised with 70 % alcohol. The albumen from all the eggs laid by each hen during one week were collected and pooled in a sterile ambience. Each pool was homogenised using an ultra-turrax T25 (Roucaire, Courtaboeuf, France) and then divided into two parts to perform bacterial and biochemical analyses.

Bacterial growth assays: after checking for sterility, the egg white coming from each hen was dispatched into two flasks (20 ml per flask) prior to inoculation with *S.* Enteritidis (a double antibiotic resistant strain at 100 µg/ml nalidixic acid and 500 µg/ml streptomycin, INRA, Tours) or *S. aureus* (wild hen strain, ENSA, Rennes). The bacteria were prepared in a filtrate of egg white (obtained by ultra-filtration, cut off point of 10000 Da), then inoculated at a concentration of 2% and homogenised with an ultra-turrax to obtain a final inoculum of about 10^3 cells/ml of egg white. This

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inoculum was controlled by plating on TSA (Tryptone Soy Agar, Biomérieux, Marcy-l'étoile, France) for *S. aureus* or TSA containing 100 µg/ml nalidixic acid and 500 µg/ml streptomycin for *S.* Enteritidis. The inoculated albumens were dispatched in one-ml tubes and incubated at 30 °C for one and 5 day(s) for *S.* Enteritidis and 8 days for *S. aureus*. After incubation, tubes were removed and examined for *S*. Enteritidis or *S. aureus* cell counts. Serial decimal dilutions in Tryptone salt were prepared and samples of each dilution were plated onto TSA or TSA containing antibiotics, depending on the bacterium. After incubation at 37°C for 24-28 h, the colony forming units (cfu) were counted and transformed in log cfu.

Quantification of egg white lysozyme: we optimized an indirect ELISA (Enzyme-Linked Immunosorbent Assay) to quantify lysozyme in egg white. All incubations were performed at ambient temperature under mild agitation, except for the incubation of the mix antigen/primary antibody (37°C). Between each step, 96-well polystyrene microtiter plates (F96 certified Maxisorp, Nalge Nunc International, Rochester, US) were washed 4 times with PBS (phosphate buffered saline: 0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride, pH 7.4). Volumes were 50 µl/well except during washing (100 µl/well). The day before the assay, different dilutions of chicken egg white lysozyme (Ovonor, Annezin-lès-Bethune, France) were prepared in TPBS-gel (PBS, 0.05 % Tween 20, 1 % gelatine, pH 7.4) for the calibration curve. Albumens to analyse were diluted 1/3000 in the same buffer. An equal volume of rabbit polyclonal anti-(chicken lysozyme) antibody -AC_1- (Biodesign, Saco, US) diluted 1/650 in TPBS-gel was then added to the dilutions of standard lysozyme and egg white (final AC_1 dilution of 1/1300). Controls corresponding to 0 % and 100 % of inhibition were prepared with TPBS-gel/AC_1 and TPBS-gel, respectively. Mixes were incubated at 37°C for 18 h under agitation. The next day, they were deposited onto the plates previously coated 2 h with chicken egg white lysozyme at 0.02 µg/ml in PBS, and incubated 1h30. Plates were then washed and incubated 2 h with donkey anti-(rabbit IgG) peroxidase-linked antibody -AC_2- (Amersham Pharmacia Biotech, Uppsala, Sweden) diluted 1/5000 in TPBS-gel. After a final washing, a peroxidase substrate, ortho-phenylenediamine (OPD) at 0.4 mg/ml in 0.05 M phosphate-citrate buffer with urea hydrogen peroxide (kit Sigma Fast, Sigma, St-Louis, US), was added in each well. After an incubation of 30 mn, the reaction was stopped with 1 M H_2SO_4. Absorbance was read at 490 nm using a microplate reader. Lysozyme concentrations of egg white samples were determined after fitting the standard curve with the four-parameter logistic equation (GraphPad Prism, version 3.02 for Windows, GraphPad software, San Diego, US). Lysozyme quantity was expressed in mg/ml and in percentage relative to egg white total proteins content, previously measured by the method of Bradford (1976).

Statistical analyses: they were performed using SAS software (SAS Institute, 1989). After checking normality (PROC UNIVARIATE), each parameter was submitted to a variance analysis to test age and hen effects within each period (PROC GLM). For

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each age, Pearson and Spearman correlation coefficients were estimated (PROC CORR). Using PROC MIXED, each parameter was explained by a fixed effect of the age and a random effect of the hen, variance of which is noted σ^2h, and a residual random effect, variance of which is noted σ^2r. The repeatability r (Ollivier, 1981) was estimated as follows:

$$r = \frac{\sigma^2 h}{\sigma^2 h + \sigma^2 r}$$

Results and Discussion

Total proteins contents correspond to that usually encountered in the literature whereas lysozyme contents are slightly inferior (10-11 % by weight of whole albumen and 3.4 % of total proteins, respectively, in Burley and Vadehra, 1989) (table 1). Between-hens variability was mainly evidenced during the first period for these two parameters (table 2). Such hen effect on lysozyme content was already described by Wilcox and Cole (1954). A decline in the albumen protein amount as well as albumen solids and lysozyme content with hens' laying period is usually reported (Wilcox, 1956; Cunningham et al., 1960; Rose et al., 1966). However, we observed the opposite in our experiment. Thus, a study concerning older hens would be useful to confirm this trend. Whatever the period, we observed a low growth of *S.* Enteritidis compared to what is usually obtained in an optimum medium (TSB: Tryptone Soy Broth) with an inoculum of a similar size (Baron et al., 1997) (table 1). These results agree with those of several authors reporting the bacteriostatic effect of albumen on *Salmonella* (Lock and Board, 1992; Baron et al., 1997). For *S. aureus*, a bactericide effect of the albumen was evidenced (table 1). According to Ng and Garibaldi (1975), this effect could be due to lysozyme. This could explain the slightly negative significant correlation between lysozyme content (in mg/ml) and *S. aureus*' growth for 24-week-old hens. However, as a general manner, no significant correlation between lysozyme content and bacterial growth was evidenced, though the amount of this protein does not obligatorily reflect its anti-bacterial activity. This supports the action of other anti-bacterial proteins against *S.* Enteritidis and *S. aureus*, such as ovotransferrin (Burley and Vadehra, 1989). As a general manner, repeatabilities were good for bacterial growth (except for *S. aureus* for the first period) and relatively low for total proteins and lysozyme contents, especially during the second period (table 3). Thus there is a need to study other albumen anti-bacterial proteins. With this aim in view, we are currently quantifying ovotransferrin. To further estimate heritabilities, we will concentrate on the global anti-bacterial action of the albumen. Thus, the second period of laying will be considered because: 1- both bacteriostatic and bactericide effects of the albumen

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are more important in this period than during the first one, 2- repeatabilities are good for both bacterial inhibition growths, 3- between-hen's variability (hen effect) is significant at the same time for *Salmonella* d1 and d5 and for *Staphylococci*.

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Table 1. Average values (± Standard Deviation) and intra-age correlations[1] of total proteins content, lysozyme content, *S.* Enteritidis' growth 1 or 5 day(s) post-inoculation and *S. aureus*' growth 8 days post-inoculation.

Age[2]	Prot. (mg/ml)	Lyso. (mg/ml)	Lyso. (%)	Sal. d1 (log cfu)	Sal. d5 (log cfu)	Staph. d8 (log cfu)
22	109.9 (11.2) #(-) ψ(+)	2.9 (0.4) ψ(+)$(+)	2.7 (0.4) #(-)$(+)	4.3 (0.8) £(+)	5.1 (1.5) £(+)	4.1 (0.3)
23	111.5 (10.1) -	2.8 (0.4) -	2.5 (0.4) -	- -	- -	- -
24	108.6 (10.3) #(-)	2.9 (0.5) $(+)&(-)	2.7 (0.5) #(-)$(+)	4.7 (0.6) £(+)	5.7 (0.8) £(+)	3.1 (0.7) &(-)
25	110.4 (9.6) #(-)	2.9 (0.5) $(+)	2.6 (0.5) #(-)$(+)&(+)	4.6 (0.6) £(+)	5.6 (1.1) £(+)	2.7 (0.6) &(+)
35	113.5 (9.8) #(-)§(+)@(+)	2.8 (0.4) $(+)	2.5 (0.4) #(-)$(+)	3.9 (0.6) £(+)	3.9 (1) §(+)£(+)¥(+)	2.8 (0.8) @(+)¥(+)
36	115.4 (9.3) #(-)	2.9 (0.4) $(+)	2.6 (0.4) #(-)$(+)	4.1 (0.5) £(+)	4.4 (1) £(+)	2.2 (0.6)
37	114.6 (8.8) #(-)	2.9 (0.5) $(+)	2.6 (0.4) #(-)$(+)	5.2 (1.1) £(+)€(+)	5.5 (1.2) £(+)¥(+)	2.9 (1.3) €(+)¥(+)
38	113.4 (7.6) #(-)ψ(+)	3.1 (0.4) ψ(+)$(+)	2.7 (0.4) #(-)$(+)	3.3 (0.4) £(+)€(+)	2.9 (0.9) £(+)¥(+)	2.3 (0.4) €(+)¥(+)

[1]a same symbol is attributed to significantly correlated parameters ($P \leq 0.05$). Sign of the correlation is bracketed. [2]week 23 was not taken into account for bacterial counts, following an inoculation problem.

Table 2. Hen and age effect within each period, and period effect for total proteins content, lysozyme content, *S.* Enteritidis' growth 1 or 5 day(s) post-inoculation and *S. aureus*' growth 8 days post-inoculation.

Period	Effect[1]	Prot.	Lyso.	Lyso.%	Sal. d1	Sal. d5	Staph. d8
22-25	**Hen**	***	***	0.115	***	0.151	0.272
	Age	0.204	0.084	*	***	**	***
35-38	**Hen**	0.061	*	0.113	***	***	***
	Age	0.320	0.249	0.068	***	***	***
	Period	***	*	0.103	***	***	***

[1]significant effects are noted *, ** and *** for $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively.

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Table 3. Repeatabilities within each period for total proteins content, lysozyme content, *S.* Enteritidis' growth 1 or 5 day(s) post-inoculation and *S. aureus*' growth 8 days post-inoculation.

Period	Prot.	Lyso.	Lyso.%	Sal. d1	Sal. d5	Staph. d8
22-25	0.22	0.20	0.08	0.32	0.07	0.10
35-38	0.15	0.11	0.10	0.29	0.27	0.38

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THE EFFECT OF ADDING COMMERCIAL FEED ENZYMES TO WHEAT-BASED FEEDS ON EGG QUALITY IN LAYING HENS

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Keywords : laying hens, enzymes, egg quality, AME, viscosity

Abstract

In Trial 1, 4 enzymes were added to wheat-based layer diets and fed to Isa Brown laying hens from 25 weeks of age. In Trial 2 (50 to 65 weeks of age), all diets were based on wheat or wheat containing 20% cereal rye. Egg quality, apparent metabolisable energy (AME) and excreta moisture were measured every 5 weeks. The AME of the diets was similar for the diets in both trials. Excreta moisture was not affected by enzymes in either trial. Egg quality varied with hen age and was significantly better for normal wheat than pinched wheat and for wheat+rye versus wheat. There were some effects of enzymes on egg quality. Production was not affected by type of diet or enzymes in either trial. Digesta viscosity was not affected by the addition of enzymes but was greater when rye was added.

Résumé

Lors d'un premier essai, 4 enzymes ont été ajoutées à des régimes à base de blé pour poules pondeuses. Des poules IsaBrown ont été nourries avec ces régimes à partir de l'âge de 25 semaines. Dans un 2^{d} essai (poules âgées de 50 à 65 semaines), tous les régimes sont à base de blé ou de blé associé à 20% de seigle. La qualité des œufs, l'énergie métabolisable apparente (AME) et la teneur en eau des fèces ont été mesurées toutes les 5 semaines. Nous avons obtenu des AME similaires pour les régimes des 2 essais. La teneur en eau des fèces n'a pas été affectée par les enzymes, quel que soit l'essai. La qualité des œufs a varié avec l'âge des poules et s'est avérée supérieure pour le blé normal par comparaison avec du blé échaudé ou pour le blé associé au seigle par comparaison au blé seul. Nous avons observé quelques effets des enzymes sur la qualité des œufs. La production d'œufs n'a pas été affectée par le type

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de régime ou les enzymes, quel que soit l'essai. La viscosité des digesta n'a pas été modifiée par l'addition d'enzymes mais a augmenté en présence du seigle.

Introduction

Enzymes are added to commercial layer diets to increase the digestibility of feed ingredients, reduce the incidence of wet droppings resulting from non-starch polysaccharides in the diets (Acamovic, 2001) and to increase the availability of feed microingredients which influence egg shell quality (Hurwitz, 1987). A recent study showed that addition of commercial enzyme preparations improved egg shell quality in wheat- and barley-based layer diets but that there were some negative effects on shell colour and Haugh Units (Roberts and Choct, 1999; Roberts et al., 1999). In Australia, wheat is a common ingredient in layer diets. However, the quality and composition of Australian wheats are variable (Choct and Hughes, 1996). The present study was therefore conducted to investigate the effect of dietary enzymes and wheat quality on egg and egg shell quality in Isa Brown laying hens.

Material and Methods

For Trial 1, two basal diets were formulated to standard commercial specifications, each containing 670 g/kg of either "normal" wheat or "pinched" wheat (water-stressed prior to harvest). The other ingredients were identical in the two diets. The basal diets were each used to prepare five experimental diets by adding one of four commercial feed enzyme preparations according to the manufacturers' instructions; a control diet of each wheat type (no enzyme added), Biofeed Wheat (175 g/tonne), Avizyme 1302 (265 g/tonne), Roxazyme G2 granular (100 g/tonne), or Kemzyme W dry (600 g/tonne). For Trial 2, all diets were based on "pinched" wheat and, for the birds that had previously received the "normal" wheat, 20% of the pinched wheat was substituted with cereal rye. In Trial 1, the diets were fed from 25 to 50 weeks of age to 760 Isa Brown laying hens which were maintained, three to a cage, in a commercial poultry house at the University of New England "Laureldale" Poultry Farm. The different treatment groups were randomised to avoid effects due to position in the poultry house. Trial 2 was conducted on the same birds at 50-65 weeks of age.
Egg and egg shell quality were assessed at 27 and 30 weeks of age, then at 5 weekly intervals. At each age, 300 eggs were collected, 30 from each of the ten treatment groups. Egg and egg shell quality analyses were completed within 24 hours of the eggs being laid. Measurements taken to assess egg shell quality were egg weight,

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shell reflectivity (an indication of the colour of the egg shell), egg shell breaking strength (measured by quasi-static compression), deformation (the distance that the egg shell is depressed by the shell breaking strength machine before the shell cracks) and shell weight (Technical Services and Supplies, U.K.). The percentage shell was calculated as the ratio of shell weight to egg weight, expressed as a percentage. The internal quality of the eggs was assessed as albumen height and Haugh Units as well as yolk colour.

Apparent metabolisable energy (AME) and excreta moisture were measured every 5 weeks from 30 to 65 weeks of age. AME was determined by the conventional total collection procedure. Birds had received the experimental diets for at least 5 weeks prior to the AME assays which were conducted over 4 days. Feed intake was measured and all excreta collected daily. Excreta were dried in a fan-forced oven at 80ºC for 36 h and excreta from each replicate were pooled over the collection period for the determination of gross energy (GE). AME of diets was calculated as:

$$\frac{(\text{g of feed eaten} \times \text{GE of feed}) - (\text{g excreta voided} \times \text{GE excreta})}{\text{g feed eaten}}$$

At the end of Trial 2, digesta were collected from 5 birds from each diet, centrifuged and the viscosity of the supernatant determined using a Brookfield DVIII Model viscometer.

Data were analysed by ANOVA with bird age, wheat type and enzyme treatment as independent variables. Differences between means were assessed by Fisher's (Protected) Least Significance Difference test. Significance was assumed at $P<0.05$.

Results and discussion

Contrary to expectation, on analysis the two wheats used in Trial 1 were found to be similar for total, soluble and insoluble non-starch polysaccharides. Protein analysis of both wheats and the control diets prepared from them showed that the crude protein level was higher for pinched wheat (178 g/kg in the wheat, 228 g/kg in the final diet) than for normal wheat (149 g/kg in the wheat, 185 g/kg in the final diet). However, the average AME of the two diets from 30 to 50 and 50-65 weeks of age was similar. AME increased to 40 weeks of age ($p<0.0001$) and remained relatively constant (Table 1).

Feed intake and excreta moisture of birds on the two wheat diets in Trial 1 and the wheat and wheat+rye in Trial 2 were also similar, as was production. The addition of commercial enzyme preparations had no significant effect on AME, excreta moisture or production in either trial (data not shown).

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At the end of Trial 2, digesta viscosity was significantly higher for the wheat+rye diet than for the wheat diet in both the jejunum (wheat 1.95 cP, wheat+rye 3.19 cP) and the ileum (wheat 3.57 cP, wheat+rye 8.88 cP) but was not affected by the addition of commercial enzyme preparations.

Egg and eggshell quality, in general, deteriorated with the age of the hens (data not shown). Egg weight and shell weight increased although the increase in shell weight was not proportional to that of egg weight, resulting in a reduction in the percentage shell. Shell thickness decreased from 27 to 35 weeks of age and then increased. Haugh Units deteriorated with increasing hen age, and yolk colour increased overall. The age-related changes in egg and eggshell quality are similar to those reported previously.

In Trial 1, eggs laid by birds receiving diets based on normal wheat had greater shell breaking strength, Haugh Units, percentage shell, shell weight and shell thickness, and darker shell colour than those given pinched wheat (Table 2). For Trial 2, pinched wheat containing 20% cereal rye resulted in better egg quality than the diet containing only pinched wheat (Table 3). The type of grain on which diets are based therefore appears to have had direct effects on egg quality

Enzyme type and/or inclusion had significant effects on shell breaking strength, shell reflectivity, yolk colour, % shell and shell thickness in Trial 1 and shell colour and albumen quality in Trial 2 (Table 4). Shell breaking strength was lower on diets with Biofeed Wheat or Avizyme than for the control, Roxazyme and Kemzyme. Shell reflectivity was slightly but significantly higher for diets with Roxazyme or Kemzyme. Percentage shell and shell thickness were highest for Kemzyme whereas yolk colour varied, being highest for the control and lowest for Avizyme.

The improved shell breaking strength observed in a previous study in eggs from birds given enzymes (Roberts and Choct, 1999; Roberts et al., 1999) was not found in the present study. This may be due to differences in feed ingredients used in the two experiments. However, the decrease in shell colour in response to dietary enzyme inclusion, was consistent across the two studies. Percentage shell and shell thickness were improved only by Kemzyme in Trial 1. However, Kemzyme had negative effects on shell colour and albumen quality during Trial 2. Yolk colour was slightly lower than the control for all diets containing enzymes in Trial 1. However, all treatment groups had yolk colour at very acceptable levels.

In conclusion, both type of wheat and the addition of commercial feed enzyme preparations had effects on egg and egg shell quality.

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Table 1. Effect of hen age on AME (MJ/kg DM) For Trials 1 and 2

Age (wks)	30	35	40	45	50	55	60	65
AME	^{c}13.0 ±0.1	^{c}13.0 ±0.1	^{a}14.4 ±0.1	^{a}14.0 ±0.1	^{b}13.5 ±0.1	^{b}13.3 ±0.1	^{b}13.5 ±0.2	^{b}13.8 ±0.2

Means with no common superscript differ significantly ($P<0.05$)

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Table 2. Effect of wheat type on egg and egg shell quality in Trial 1

Wheat Type	B.S. N	Reflect %	% Shell	Shell Wt g	Thickness µm	Haugh Units
Normal	[a]38.3 ±0.2	[b]31.8 ±0.1	[a]9.45 ±0.03	[a]6.14 ±0.02	[a]405.3 ±0.9	[a]93.4 ±0.3
Pinched	[b]36.6 ±0.2	[a]32.2 ±0.1	[b]9.34 ±0.02	[b]6·07 ±0.02	[b]399.6 ±0.9	[b]92.0 ±0.2

Means within columns with no common superscript differ significantly (P<0.05)

Table 3. Effect of diet (wheat or wheat+rye) on egg and egg shell quality in Trial 2

Diet	B.S. N	Shell Wt g	% Shell	Albumen Ht mm	Haugh Units	Yolk Colour
Wheat	[b]32.5 ±0.4	[b]6.23 ±0.03	[b]9.05 ±0.04	[b]7.98 ±0.07	[b]85.7 ±0.5	[a]11.79 ±0.3
Wheat+ rye	[a]33.9 ±0.4	[a]6.35 ±0.03	[a]9.20 ±0.04	[a]8.36 ±0.06	[a]88.4 ±0.4	[b]11.65 ±0.4

Means within columns with no common superscript differ significantly (P<0.05)

Table 4. Effect of enzyme treatment on egg and egg shell quality in Trials 1 and 2

Trial	Measure-ment	Control	Biofeed Wheat	Avizyme	Roxazyme	Kemzyme
1	B.S. N	[a]37.8 ±0.03	[b]36.6 ±0.3	[b]36.8 ±0.4	[a]37.8 ±0.4	[a]38.2 ±0.4
	Reflect %	[c]31.6 ±0.2	[c]31.4 ±0.2	[bc]31.8 ±0.2	[b]32.0 ±0.2	[a]33.2 ±0.3
	% Shell	[b]9.35 ±0.04	[b]9.30 ±0.03	[b]9.35 ±0.04	[b]9.40 ±0.04	[a]9.57 ±0.04
	Shell Thickness µm	[b]401.5 ±1.4	[b]400.0 ±1.3	[b]401.3 ±1.5	[b]402.3 ±1.7	[a]407.3 ±1.5
	Yolk Colour	[a]11.48 ±0.05	[b]11.25 ±0.05	[c]11.03 ±0.05	[b]11.27 ±.05	[b]11.35 ±0.5
2	Reflect %	[b]34.1 ±0.4	[b]34.3 ±0.4	[b]34.6 ±0.4	[b]34.9 ±0.4	[a]36.1 ±0.4
	Albumen Ht mm	[a]8.31 ±0.12	[a]8.35 ±0.09	[a]8.09 ±0.11	[a]8.36 ±0.10	[b]7.74 ±0.12
	Haugh Units	[a]87.7 ±0.8	[a]88.4 ±0.6	[a]86.6 ±0.8	[a]88.3 ±0.6	[b]84.5 ±0.8

Means within rows with no common superscript differ significantly (P<0.05)

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INCUBATION TEMPERATURE FOR OSTRICH EGGS

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Keywords: Ostrich, incubation, temperature , embryo mortality, hatchability

Abstract

The impact of incubation temperature on hatchability, egg weight loss and embryonic mortality of 540 ostrich eggs were studied. Eggs were obtained from three farms that used different sex ratios for mating. Either 97.5, 98.5 or 99.5 °F incubation temperature with relative humidity ranged from 20 to 30% were used. Results show that the lowest temperature used improved hatchability of fertile eggs, increased hatching time and significantly decreased the incidence of early dead, late dead, dead in shell and total dead embryos in comparison to the other treatments. These results show that the most effective incubation temperature for the ostrich is lower than that used for most birds.

Résumé

L'influence de la température d'incubation sur l'éclosion des œufs, la perte de poids des œufs et la mortalité embryonnaire de 540 œufs d'autruche étaient étudiée. Les œufs provenaient de 3 fermes utilisant differents ratio mâle/femelle pour la reproduction. Des températures d'incubation de 36.39, 36.94 ou 37.5 °C et des humidités relatives de 20 et 30% étaient utilisées. Les résultats montrèrent que l'éclosion des œufs fertiles, le temps d'éclosion et le taux de mortalité des embryons étaient meilleurs à la plus basse température. Les résultats montrèrent que la température la plus efficace pour les autruches est plus faible que celle utilisée pour la majorité des oiseaux.

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Introduction

The increasing demand for ostrich meat in some parts of the world has ostrich breeder's pressure on accelerates production. Low reproduction efficiency has focused attention on artificial incubation. Hatchability of ostrich eggs incubated in different countries is highly variable. Deeming (1995b) reports that artificial incubation is an essential part of any commercial ostrich farming enterprise. In general, hatchability of ostrich eggs in artificial incubators is considerably below that achieved in natural nests in the wild (Burger and Bertram, 1981). The work of Burger and Bertram (1981), Jarvis et al., (1985) and Swart (1988) who studied temperature and humidity conditions of naturally incubated ostrich eggs improved artificial incubation on ostrich farms.

Incubation conditions that yield high hatchability in a variety of bird species indicate that attaining a water loss of 13-15% of initial egg mass by the time of complete embryo development is optimal. However, temperature and humidity relationships have never been successfully standardized that consistently yield healthy chicks as is standard in the chicken, turkey and duck industries, and other species (Ar, 1991). Cause and effect relationships cannot be correctly attributed when temperature and relative humidity are not optimized scientifically for standard embryo deveopment and egg water loss. Embryo developmental rate and internal embryo temperature must be studied to establish optimal temperature for embryonic development.

Material and Methods

A total of 541 ostrich (Struthio camelus) eggs were collected from February till August during 2002 from three ostrich farms, which were between 1 and 25 day old when set in the incubator. Eggs were weighed (_+1g). Eggs were incubated and hatched in ostrich egg setting racks or hatchers basket. Eggs from each farm were randomly distributed among 3 incubation temperatures, either 36.39, 36.94, or 37.5 C from 1 to 38 d of incubation and 36 thereafter during that hatching period which extend from 39 to 44d. Relative humidity ranged from 20 to 30% RH fro the start of incubation until hatch was complete. Eggs were turned automatically through an angle of 45 degree once each four hours. Eggs were examined 14 d after the start of incubation and every 7 d thereafter. Each egg was candled and weighed weekly starting from 7 d to determine egg fertility and weight loss. Infertile eggs were removed day 21 and opened to cofirm lack of embryonic development. At regular intervals during the hatching period eggs were observed whenever possible to record the time of external pipping, and successful hatching was recorded. Unhatched eggs

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were classified as infertile, early dead, late dead or dead in shell embryos. Chicks were weighed as soon as possible after hatching and hatching time was recorded.Statistical analysis: Data were analyzed using a two- way analysis of varience. Proportional data were transformed to arcsine before analysis. Differences among treatments were separated by Duncan's New Multiple Range Test (Duncan, 1955).

Results and discussion

General observations:

No different significant was found among eggs that used in the present study whether in the fertility or initial egg weight or egg storage period among the treatments. While fertility was 57.58+,44.03+ and 57.95+% , initial egg weight was 1502.26+9.78, 1530.90+15.03 and 1521.09+11.75g and egg storage period was 8.06+0.37, 7.74+0.54, and 7.91+ 0.44d for eggs incubated at 36.39, 36.94, and 37.50 C, respectively). No significant interaction was found between treatment and farms at any trait.Incubation performance: Hatchability of fertile eggs was significantly higher (72.18+%) for eggs incubated at 36.39C as compared with either (33.89+ or 18.63+%) for eggs incubated at 36.94 and 37.50C, respectively). However, no significant differences in the hatchability of fertile between the eggs incubated in 36.94 and 37.5 C (33.89+ and 18.63+%, respectively). Hatchability of the eggs incubated in 37.5 C was the lowest one. While Foggin and Honywill (1992) in Zimbabwe found that hatchability of eggs set was 3.3%.Mellett (1993) in South Africa found that an average 50% of all eggs hatch. Deeming et al. (1993) in Great Britain (Namibia) found that hatchability of set and fertile eggs was 60 and 69.2%, respectively. Wilson et al. (1997) in USA found that hatchability of fertile eggs was 66%. Cloete et al. (1998) in South Africa found that hatchability of set and fertile eggs was 46.2 and 56.4%, respectively. This result may reflect fundamental differences between eggs from different strains of ostrich but it is more likely that factor such as egg quality and incubation conditions especially the incubation temperature are adversely affecting hatchability rates.

Egg weight loss :

Egg weight loss percentage at 28d of incubation was not varied significantly among the treatments (9.00+,8.26+ and 8.98+% for eggs incubated under 36.39, 36.94, and 37.5 C, respectively). Also, egg weight loss percentage at 38d was not varied significantly among the treatments (12.09+,11.77+ and 12.18+% for eggs incubated under 36.39, 36.94, and 37.5 C, respectively). Ar and Rahn (1980) found that, average 15% of the initial egg weight is lost during incubation. Christensen et al. (1996) noted that, the optimal incubator humidity for ostrich eggs is less than 25% to allow a 15% loss of initial egg mass during the 45 d incubation period. The mean weight losses of 13-14% being recorded (Burger and Bertram, 1981, Jarvis et al., 1985, Swart et al.,

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1987, Deeming et al., 1993).The results of the study showed that incubator humidity and egg weight loss of ostrich eggs was within the recommended level and the main factor affecting the hatchability was the incubation temperature. In addition, incubation temperatures need to be adjusted (36.36 C or less) to allow a longer development time for the embryo to attain an adequate level of maturity to survive the plateau stage in oxygen consumption or to prevent the use of limited energy of the yolk to survive the anoxia of tucking and internal piping. The optimal incubation temperature of ostrich eggs appears to be between 36.39C and less. In the present study, the low relative humilities recorded in the incubator, and the good average weight losses achieved indicated that the main factor affecting hatchability and embryonic mortality is the incubation temperature.

Embryonic Mortality :

Eggs incubated at 36.39 C decreased significantly the early dead embryo (EDE) 6.77+% as compared with both 36.94 C (13.56+%) and 37.5 C (16.67+%). However, the difference between 36.36 and 37.5 C were no significant. There was a considerable variation in late dead embryo (LDE) among eggs incubated at 36.39 C (1.50+%) and 36.94 (5.09+%) and 37.5 C (10.78 +%).Eggs incubated at 36.39 C decreased significantly the dead in shell (DIS) (19.55+%) as compared with both eggs incubated at 36.94 (47.46+%) and 37.5 C (53.92+%). The present study confirms earlier observations (Deeming et al., 1993) that it applies to ostriches. Deeming (1995) in Zimbabwe found that, the embryonic mortality was high at the start and end of incubation, a pattern similar to that of other domestic birds. Mortality of late stage embryos was related to percentage water loss with extremes of the ranges causing the highest mortality. The humidity regimen of the incubator in conjunction with shell porosity and temperature incubation has, however, been shown to play an important role in explaining some of the mortality of these embryos. Peaks of the mortality were observed during the first and last weeks of incubation.

Conclusions

Additional research is needed to study the effect of lower incubation temperature than 36.39 C on the hatchability and embryonic mortality. Also we need to study the relationships among incubation temperature, egg shell quality, hatchability, embryonic mortality and egg weight loss.

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INFLUENCE OF OVIPOSITION TIME AND LIGHTING PROGRAMME ON EGGSHELL QUALITY

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Keywords : Eggshell Quality, Oviposition Time, Midnight Lighting Program, Laying Hens

Abstract

The times of oviposition are affected little by the use of a night lighting programme. Egg shell weight is not changed by the time of oviposition. Egg weight, shell percentage and it's colour vary according to the time of oviposition. The egg characteristics have not been affected by the use of night lighting. Night lighting reduces the percentage of eggs downgraded, in particular reducing by 37 % the percentage of eggs with pimpled ends.

Résumé

Les heures d'oviposition sont peu affectées par l'utilisation d'un éclairement de nuit. Le poids de coquille n'est pas modifié par l'heure d'oviposition. Poids de l'œuf, pourcentage de coquille et couleur varient en fonction des heures d'oviposition. Les caractéristiques de l'œuf n'ont pas été affectées par l'utilisation de l'éclairement de nuit. L'éclairement de nuit réduit le pourcentage d'œufs déclassés, en particulier réduit de 37 % le pourcentage d'œufs à bouts granuleux.

Introduction

Previous research on oviposition times of laying hens is relatively dated. We wanted in this experiment to evaluate the influence of night lighting on oviposition times and to determine exactly the oviposition times. The egg characteristics were then measured as a function of this oviposition time.

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Materials and Methods

The experiment was carried out at the INZO° experimental station. At 51 weeks of age, 1152 ISABROWN pullets were allocated into 4 rooms of 288 birds. In 2 rooms, the duration of the light period remained at 15 hours with "lights on" at 4.00h and "lights off" at 19.00h. The other 2 rooms received night lighting from 22.45h to 0.15h. In the 56 and 61th week all eggs laid during one day were collected at specified times. Shell quality was measured on the 1817 intact eggs collected. Egg weight, shell deformation and breaking force were measured using an MTS compression apparatus and shell colour was evaluated using a MINOLTA apparatus. For shell colour the L-a-b values were measured. Shell weight and percentage of shell were also measured. Over the period 53-61 weeks, the downgraded eggs were divided into 2 categories, eggs with pimpled shells and cracked eggs.

Results and discussion

Influence of night lighting

The length of night was 9 hours; the first egg collection was carried out at 12 minutes after "lights on". The estimated times by which the different rates of lay were reached are given in Table 1. The estimated times are given for all eggs collected at 56 and 61 weeks. The effect of midnight lighting on the oviposition times has been evaluated in rooms receiving or not the midnight lighting. The values ara an average of the egg collections made at 56 and 61 weeks.

Table 1: Estimated times after "lights out" by which different rates of ovipositions were observed, during a days collections carried out at 56 and 61weeks, with and without night lighting.

Age	56 weeks	61 weeks	56 and 61 weeks	
Midnight lighting	With and Without	With and Without	With	Without
Eggs collected	952	879	922	909
10%	9h18	9h24	9h16	9h26
20%	9h43	9h48	9h42	9h49
50%	11h15	11h27	11h31	11h12
80%	12h50	13h10	13h15	12h45
90%	13h48	14h18	14h18	13h48
95%	15h05	16h14	16h03	15h16
99%	Around 19 hours after "lights off"			

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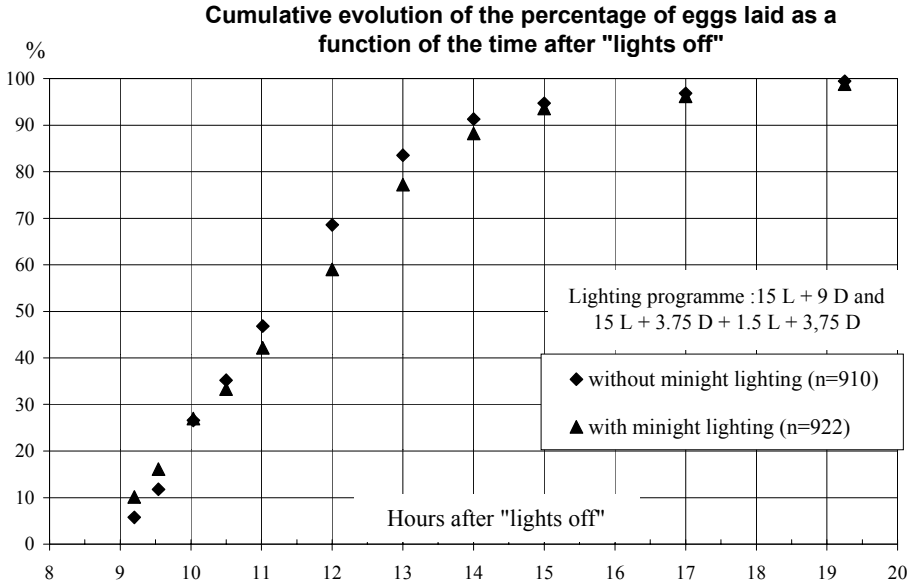
About 40% of the eggs were laid in the 2 hours which followed "lights on", which means that for at least 40% of the eggs, calcification was completed by "lights on". The cumulative percentage of the eggs collected by different intervals from "lights off" is given in the graph below.



A period of 1h30 duration light in the middle of the night had little effect on the times of oviposition. Night lighting did not change the time of the first ovipositions, on the other hand, it slightly reduced the hourly rate of ovipositions.
Monitoring of the oviposition times on other flocks showed that 10% lay was obtained by 8h17 after "lights off" (Joly 2001). These results were obtained with a light duration of 16 hours and on flocks generally at start of lay. According to Lewis (1995), oviposition times are delayed by about 30 minutes when the duration of lighting is reduced by one hour. The ovipostion times in this trial would only be delayed by about 30 minutes according to these earlier observations. This delay could be attributed to the bird age.
The physical characteristics of eggs collected at 56 and 61 weeks, were not changed by using a period of night lighting as shown in Table 2.

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Table 2 : The influence of a period of night lighting of 1h30 duration on egg characteristics (average of eggs collected at 56 and 61weeks of age).

Mid-night lighting	Number of eggs collected	Egg weight (g)	Shell weight (g)	Colour (L-a-b)	% of shell	Deformation (N/mm)	Shell strength (N)
With	946	63.57	6.44	27.1	10.15	196	35.5
Wthout	871	63.65	6.47	27.2	10.18	197	35.7
Average	1817	63.61	6.45	27.1	10.16	196	35.6

These results are, however, in contradiction to the results obtained by Grizzle et al (1992) who observed an improvement in specific gravity of the egg when a period of night lighting of 2 hours was used. Altan et al (2000) also obtained an increase in shell weight per unit area.

During the 8 weeks (53 to 61 weeks), from a total production of 52336 eggs, those with pimpled shells or cracks were counted. The results are presented in Table 3.

Table 3 : The influence of night lighting on the percentageof eggs with pimpled ends and the total seconds.

Shell defects	Pimpled ends		Total seconds	
Midnight light	with	without	with	without
53-54 weeks	1.76 A	2.74 B	4.04	4.56
55-56 weeks	1.79 A	3.03 B	4.79 A	5.67 B
57-58 weeks	2.51	2.91	5.93	6.07
59-60 weeks	1.65 A	3.85 B	4.96 A	7.13 B
53-60 weeks	1.94 A	3.10 B	4.96 A	5.80 B

For each shell defect means within a row with no common letter differ significantly ($P<0.01$)

Night lighting reduced the percentage of downgraded eggs in a highly significant manner. This reduction was due to a reduction of 37% in the number of pimpled (sandy) ends. The percentage of cracks and haircracks was not affected by the night lighting.

The influence of oviposition time on the egg characteristics.

The eggs were collected each hour with the exception of the first collection which was carried out 12 minutes after "lights on". Egg weight varied by more than 5g between the eggs laid first and last in the day. Shell weight does not appear to be affected by oviposition time. It follows that the eggs laid later have a higher percentage of shell, a better colour and a higher rupture force. The results are given in Table 4.

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Table 4: The influence of oviposition time on egg characteristics (average of 2 days collections carried out at 56 and 61 weeks)

Period of Collection	Eggs collected	E.W (g)	S.W (g)	Shell %	Coloration (L-a-b)	Strength (N)
Before 4.12h	138	65.96	6.4	9.72	30.4	34.6
From 4.12 to 5h	345	65.25	6.51	10.00	27.6	33.3
From 5 to 6h	325	64.52	6.49	10.07	27.5	34.8
From 6 to 7h	349	63.47	6.43	10.14	27.1	35.7
From 7 to 8h	305	62.83	6.41	10.22	26.7	35.6
From 8 to 9h	172	61.71	6.37	10.34	25.7	37
After 9h	185	60.51	6.5	10.75	25.3	40.1
Average	1817	63.60	6.45	10.16	27.1	35.6

The fact that the shell weight was relatively constant could mean that the quantity of calcium deposited by a hen is a genetic characteristic with little connection to egg weight. The variation in egg weight could be ascribable to the position of the egg within the clutch. The relationship between egg weight and it's place within the clutch has been recognised for a long time.

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INFLUENCE OF HENS' DIET ON EGG PRODUCTION, EGG QUALITY AND SERUM LIPID PROFILE

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Keywords : Designer egg, egg quality, serum lipids, spirulina, organic selenium

Abstract

WLH hens were fed with a control and 3 functional feeds namely FSE=150g flaxseeds + 200mg vitamin E + 3g Spirulina/ kg, FOSe=20g fish oil + 0.2mg organic selenium (Sel-plex) + 3g Spirulina/ kg and FSE+FOSe=75g flaxseed + 10g fish oil + 100mg vitamin E + 0.1mg organic selenium + 3g Spirulina/ kg, from 53 to 58 weeks of age. The hen-day egg production was significantly lower and the egg weight was higher with the FSE treatment than the rest. The albumen index and Haugh units were better in the 3 treatments than the control, where as the yolk index was lower in the control and the FOSe treatments. Hens fed on the 3 experimental feeds had significantly lower total serum cholesterol, VLDL and LDL cholesterol and triglycerides, with higher HDL cholesterol than the control.

Resumé

Des poules white Leghorn de 53 à 58 semaines ont été nourries avec un régime témoin et 3 régimes expérimentaux nommés FSE (150g graine de lin + 200mg de vitamine E + 3g Spirulina/kg), FOSe(20g d'huile de poisson + 0.2mg sélénium organique (Sel plex) + 3g Spirulina/ kg) et FSE+FOSe(75g de graine de lin + 10g d'huile de poisson + 100mg de vitamine E + 0.1mg sélénium organique + 3g Spirulina/ kg).. La production journalière d'oeufs était significativement plus basse et le poids d'oeuf plus haut avec le traitement FSE. La hauteur d'albumen et les unités Haugh étaient meilleures dans les 3 traitements par rapport au témoin. La quantité de jaune était plus faible dans le lots témoin par rapport aux lots expérimentaux. Les poules nourries avec les aliments expérimentaux avaient un taux plus faible de cholestérol sérique, ainsi que celui des VLDL, LDL et triglycérides totaux; tandis que le niveau de cholestérol HDL était plus élevé.

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Introduction

Despite the provision of best quality protein, vitamins, minerals and MUFA, greater availability and low cost, the egg consumption in developing countries is very low, mainly due to the cholesterol scare. Hence, inorder to increase the egg consumption several workers. (Farrell, 1998; Basmacioglu *et al.,* 2001 and Narahari *et al.,* 2003) have attempted to reduce the yolk and serum cholesterol levels, by feeding special diets to hens; with various degrees of success. Anderson *et al.* (1991) studied the yolk pigmentation effect of spirulina; but its effect on yolk and serum lipid profile has not been explored. The combined effect of flax seed, fish oil, spirulina, Vitamin E and organic selenium (Sel-plex) in layer diets on egg production, egg quality and serum lipid levels have not been studied so far. Hence this work was undertaken.

Material and methods

Ninety six White Leghorn Forsgate strain hens, 53 weeks of age, were randomly housed in individual cages in an open sided cage layer house, with a floor space of 930cm^2 per hen. They were randomly divided into 16 groups (replicates) of six birds each. Four groups were randomly assigned to each of the four dietary treatments in a completely randomized design. The four dietary treatments consisted of **Control-** Standard layer mash, **FSE-**Layer mash containing 150 g ground full fat flaxseed + 200 mg supplemental vitamin E + spirulina 3 g/ kg diet, **FOSe-** Layer mash containing 20 g Anchovy fish oil + organic selenium (Sel-plex) to supply 0.2 mg selenium + spirulina 3 g/ kg diet and **FSE + FOSe**-Layer mash containing 75 g ground full fat flaxseed + 10 g Anchovy fish oil + 100 mg vitamin E + 0.1 mg organic selenium + spirulina 3 g/ kg diet. The experimental feeds were fed to the respective birds *ad libitum* from 53 to 58 weeks of age. The ingredient and chemical compositions of layer feeds are shown in Tables 1and 2, respectively.

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Table 1. Ingredient composition of the experimental layer feeds (g / kg)

Ingredient	***Control***	***Flax Seed + Vit. E (F.S.E.)***	***Fish oil + Org.Se. (F.O.Se)***	***FSE + FO Se***
Corn	300	180	277	200
Broken rice	200	205	200	230
Rice polish	100	--	30	12
Deoiled rice bran	--	100	60	85
Soybean meal	200	122	200	175
Sunflower meal	100	140	110	110
Dicalcium phosphate	10	10	10	10
Shell grit	88	87.6	87.9	87.75
Flax seed	--	150	---	75
Fish oil	--	---	20	10
Trace mineral premix	1	1	1	1
Vitamin premix	1	1	1	1
Ethoxyquin	+	+	+	+
50% Vitamin -E	--	0.4	--	0.2
Organic selenium	--	--	0.1	0.05
Spirulina	-	3	3	3

Table 2. Nutrient composition of the experimental diets

Nutrient	***Control***	***F.S.E.***	***F.O.Se***	***FSE + FOSe***
CP (g/ kg)	172.0	173.5	172.3	173.2
ME (MJ/ kg)	11.05	11.08	11.07	11.09
Lipids (g/ kg)	37.5	69.1	54.7	63.5
Calcium (g/ kg)	34.5	34.2	33.9	33.8
Total phosphorus (g/ kg)	7.1	7.4	7.2	7.6
Lysine (g/ kg)	7.6	7.4	7.6	7.5
Methionine (g/ kg)	3.1	3.1	3.1	3.0
Vitamin E (mg/ kg)	29.2	219.8	96.6	160.2
Selenium (mg/ kg)	0.090	0.085	0.293	0.196
Total carotenoid pigments (mcg/ g)	6.5	18.2	19.1	18.8

The data on body weight gain, feed consumption, mortality, egg production and egg weight were collected. Two eggs from each replicate were collected for assessing their qualities. At the end of the experimental period, blood samples were collected from two hens in each replicate, the serum was separated and the serum samples were used for estimation of total(TC), VLDL, LDL and HDL cholesterol, as well as Triglyceride (TG) levels, using the one step procedure of Wybenga *et al.* (1970). All the data

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pertaining to egg production, egg quality traits and serum lipid profile were subjected to analysis of variance for a completely randomized design for significance according to the methods of Snedecor and Cochran (1989).

Results and discussion

The effect of dietary treatments on production traits and egg quality are reported in Tables 3 and 4, respectively.

Table 3. Influence of diets on various production traits in WLH hens

Trait@	***Control***	***F.S.E.***	***F.O.Se***	***FSE + FO Se***
Body weight gain^{NS} (g)	278 ± 10.04	280 ± 11.10	302 ± 16.05	280 ± 12.10
% Hen-day egg production**	63.9^{a} ± 0.73	59.1^{b} ± 0.54	61.2^{ab} ± 0.90	63.6^{a} ± 1.26
Feed consumption^{NS} (g/ hen/ day)	96.5 ± 1.90	90.9 ± 1.14	92.0 ± 1.79	92.9 ± 1.51
% Livability^{NS}	100	100	100	100
Feed/ doz. Eggs^{NS}	1.81 ± 0.04	1.84 ± 0.03	1.80 ± 0.03	1.76 ± 0.02
Egg weight (g)**	51.7^{b} ± 0.12	54.3^{a} ± 0.36	50.8^{b} ± 0.26	51.9^{b} ± 0.37
Egg mass/hen/day (g)*	33.0^{a} ± 0.76	32.1^{a} ± 0.81	31.1^{b} ± 0.88	33.0^{a} ± 0.86

Means within each row not bearing at least one common superscript differ significantly; NS= not significant; *= Significant (P<0.05); **=Highly significant (P<0.01)

Table 4. Influence of functional feeds on WLH egg components and quality

Trait@	***Control***	***F.S.E.***	***F.O.Se***	***FSE + FOSe***
% Yolk*	30.8^{a} ± 0.18	30.5^{a} ± 0.02	29.3^{b} ± 0.08	29.2^{b} ± 0.19
% Albumen*	59.8^{b} ± 0.48	60.2^{b} ± 0.35	61.4^{a} ± 0.33	61.3^{a} ± 0.13
% Shell*	9.67^{a} ± 0.23	9.64^{ab} ± 0.10	9.59^{b} ± 0.08	9.58^{b} ± 0.09
Shape index^{NS}	72.6 ± 0.32	72.2 ± 0.58	72.2 ± 0.39	71.6 ± 0.32
Specific gravity^{NS}	1.04 ± 0.11	1.03 ± 0.10	1.03 ± 0.09	1.02 ± 0.08
Shell thickness(mm) ^{NS}	0.33± 0.02	0.33 ± 0.02	0.33 ± 0.02	0.32 ± 0.01
Albumen index*	0.10^{b} ± 0.02	0.11^{a} ± 0.03	0.11^{a} ± 0.03	0.11^{a} ± 0.03
Yolk index*	0.46^{b} ± 0.00	0.47^{a} ± 0.01	0.46^{b} ± 0.00	0.47^{a} ± 0.01
Haugh unit*	99.2^{b} ± 0.45	101.2^{a} ± 0.55	102.1^{a} ± 0.32	101.5^{a} ± 0.18
Roche yolk colour**	8.0^{c} ± 0.32	12.0^{b} ± 0.19	12.4^{ab} ± 0.18	12.9^{a} ± 0.13

Means within each row not bearing at least one common superscript differ significantly; NS= Not significant; *= Significant (P<0.05); **= Highly significant (P<0.01).

No significant differences were noticed in the body weight gain, feed consumption, feed efficiency and livability, between the four dietary treatments; indicating that the

flax seeds and the anchovy fish oil used in this study were not harmful to the birds. Simlarly, Huang *et al.* (1990) concluded that fish oil had no adverse effect on body weight gain. Galobart *et al.* (2001) observed no differences in feed consumption, feed efficiency and livability.

Egg production was significantly ($P<0.01$) lower in the FSE group and intermediate in the FOSe treatment, compared to control and FSE + FOSe combination; indicating that high levels of dietary flax and fish oil will affect egg production. This finding concurs with the earlier work of Scheideler *et al.* (1995). However Jiang *et al.* (1991), Galobart *et al.* (2001) and Novak and Sheideler (2001) observed no depression in egg production due to flax seed feeding. These differences might be due to variations in the inclusion levels, feeding period and age of the birds. Contrary to the egg production, the egg weight was significantly ($P<0.01$) in favour of high flax treatment, resulting in comparable egg mass per hen per day. However, Jiang *et al.* (1991) and Galobart *et al.* (2001) observed no such increase in egg size due to flaxseed feeding.

The yolk and shell percentages were significantly ($P< 0.01$) lower; with proportionate increase in albumin weight, due to dietary fish oil at both the levels tried (Table 4). Marshall *et al.* (1994) also observed reduction in yolk size due to fish oil feeding.

Effect of dietary treatments on various egg quality traits (Table 4) showed no significant ($P<0.05$) differences between dietary treatments on shape index, specific gravity and shell thickness. Novak and Scheideler (2001) also reported that the egg specific gravity was not affected by dietary flaxseed. The albumen index, yolk index, Haugh unit and Roche yolk colour values were influenced by dietary treatments significantly (<0.05). In all the three functional diets, the albumen quality expressed as albumen index and Haugh unit were slightly better than the control. This is in accordance with the report of Ahn *et al.* (1995), who noticed an improvement in Haugh unit due to flaxseed supplementation. The yolk index was significantly ($P<0.05$) higher in the FSE and FSE + FOSe treatments. Even though the yolk index was significantly different between treatments, it did not follow any trend based on the dietary treatments. Basmacioglu *et al.* (2001) did not notice any significant variations in the egg quality due to flaxseed supplementation; but, with fish oil supplementation, depression in Haugh unit was recorded by them.

Feeding hens with functional feeds, rich in omega-3 fatty acid has not only reduced the bad TC, VLDL-C, LDL-C and TG levels; but also increased the good HDL-C levels significantly ($P<0.01$), as shown in Table 5. Similar patterns were observed by On and Kang (1999) and Narahari *et al.* (2003) with dietary n-3 fatty acids and serum lipid profiles in hens. Previously it was thought that if the serum TG and TC in hens fall, egg production will be affected severely; but no such correlation was noticed in this study; suggesting that it is possible to produce omega -3 fatty acid rich eggs; without affecting egg production. Based on this study, it may be concluded that a combination of flaxseed with fish oil, vitamin-E, organic selenium and Spirulina will be superior for production of functional eggs, than few of these combinations.

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Table 5. Effect of function feeds on serum lipid profile of the hens (mg/dl)

Trait[@]	*Control*	*F.S.E.*	*F.O.Se*	*FSE + FO Se*
Total Cholesterol[**]	$176.4^{b} \pm 7.54$	$140.0^{a} \pm 7.44$	$144.2^{a} \pm 6.30$	$147.4^{a} \pm 5.66$
VLDL-C[**]	$115.8^{b} \pm 8.24$	$69.9^{a} \pm 6.82$	$71.5^{a} \pm 5.54$	$80.8^{a} \pm 7.42$
LDL-C[**]	$30.4^{b} \pm 1.22$	$21.9^{a} \pm 1.03$	$23.4^{ab} \pm 0.71$	$23.1^{ab} \pm 0.61$
HDL-C[**]	$30.1^{b} \pm 2.87$	$47.8^{a} \pm 0.67$	$49.4^{a} \pm 2.21$	$43.0^{ab} \pm 5.94$
TG[**]	$849.8^{b} \pm 19.59$	$715.3^{a} \pm 30.05$	$718.8^{a} \pm 19.05$	$759.0^{ab} \pm 25.43$

Means within each row not bearing atleast one common superscript differ significantly; **=Highly significant (P<0.01)

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INCREASING PERFORMANCE AND EGG QUALITY OF LAYING HENS BY PHASE FEEDING DURING TRANSITION PERIOD

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Keywords : Laying hens, Phase feeding, Performance, Egg quality

Abstract

The objectives of this experiment was to see the effect of a high protein and energy diet (PF) at onset of egg production (5%), and at peak production on performance, egg quality and follicular hierarchies of laying hens. One hundred and twenty birds were divided into five groups of treatments. Birds in the first group (T1) as control did not received PF treatment. The second group (T2) received PF when the birds reached maturity (19-20 weeks of age) then control diet. The third group (T3) received PF during peak production (27-28 weeks of age). The fourth group (T4) received PF at 19-20 and 27-28 weeks. The fifth group (T5) received PF starting from 19 to 28 weeks of ages. The PF contained 21% of crude protein and 2,850 kcal/kg ME, while control diet contained 16, 5% of crude protein and 2,750 kcal/kg ME. Each treatment was allocated to 20 hens as individual replication. The feed consumption and egg production were not affected by dietary treatment. Sexual maturity was earlier (136.85 days) for T5, accordingly the first egg weight was lighter (41.98 g) than that other treatments. High protein and energy supply increased egg weight and yolk weight whatever the period of introduction. Oviduct weight and oviduct length, ovary weight and follicle numbers were not affected by the treatments. It was concluded that high protein and energy supply on critical period of laying hens did not increased egg production and follicle numbers, but increased egg weight and yolk weight .

Resumé

Notre expérience avait pour but de connaître l'effet d'un aliment riche en énergie et protéine (FP) lors de l'entrée en production et du pic de production sur la production et la qualité des œufs et sur la croissance de l'oviducte et de l'ovaire. Cent vingt poules âgées de 19 semaines divisées en cinq groupes, T1: aliment de contrôle (16,5% de

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protéines brutes et énergie de 2,750 kcal/kg), T2: l'aliment PF à l'âge de 19-20 semaines (21% de protéines brutes et 2,850 kcal/kg) puis l'aliment contrôle. T3: l'aliment PF à l'âge de 27-28 semaines, précédé et poursuivi par l'aliment de contrôle. T4: l'aliment PF à l'âge de 19-20 et 27-28 semaines. T6: l'aliment PF à l'âge de 19-28 semaines. Les résultats de l'expérience indiquent que la consommation d'aliment et la production d'œufs n'ont pas été modifiées par l'aliment à plus forte teneur en énergie et protéine. Par contre, il augmente le poids des oeufs et celui du jaune quelque soit la période d'apport. Le poids et la longueur de l'oviducte ainsi que le nombre et le poids des follicules n'ont pas été influencés par les traitements. En conclusion, cette expérience indique qu'un aliment à plus forte teneur en protéine et énergie, consommé pendant l'entrée et/ou le pic de production n'a pas modifié la production d'œuf. Par contre il a augmenté le poids des œufs et de leur jaune. La distribution d'aliment *phase feeding* a l'âge de 19 jusqu'à 28 semaines rend la maturité sexuelle précoce et le poids du premier œuf plus léger.

Introduction

Optimisation of layer feeding should minimize egg production cost. One possibility is to optimise feeding during the critical phase between sexual maturity and peak production time to obtain the best maturity age and higher egg production. Physiological changes in the body of the layers will occur when they are ready to lay their first eggs (19 to 20 week of age) and at peak production (27-28 week of ages) when the birds require more protein and energy to support maturing ovum and egg formation. Phase feeding, by manipulating feed protein and energy content, may contribute to maximize egg production and shell quality (Ousterhout, 1981) by modifying the number of ovum under maturation at sexual maturity ages and at peak production. Our study aims to check this hypothesis.

Material and Methods

One hundred and twenty pullets (Lohman Brown Leghorn) at 13 weeks of age were fed commercial pullet diet until 19 weeks of age (table 1). At 19 weeks, the birds were divided into five groups of treatments. Birds in the first group (T1 as control did not received PF (phase feeding) treatment. The second group (T2) received PF when the birds reached maturity (19-20 weeks of age) then the control diet. The third group (T3) received PF during peak production (27-28 weeks of age) and the control diet before and after this period. The fourth group (T4) received PF at 19-20 weeks and 27-28

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weeks and the control diet at 20-26 weeks. The fifth group (T5) received PF starting from sex maturity to production peak (19 to 28 weeks of ages) then the control diet. The so called PF contained 21% of crude protein and 2,850 kcal/kg ME, while control diet contained 16, 5% of crude protein and 2,750 kcal/kg ME. Each treatment consisted of 20 hens as individual replication. At 19 weeks, 21 weeks, 29 weeks and 33 weeks of age, five hens from each group were killed for weighing oviduct and follicles the determination of hierarchies follicles (more than 0.4 cm of diameter). Data were analysed by analysis of variance following completely randomised design.

Table 1. Scheme of phase feeding treatment

Treatment	Age (week)			
	19 – 20	21 – 26	27 – 28	29 - 34
T1	Control diet			
T2	Phase Feeding	Control diet		
T3	Control diet		Phase Feeding	Control diet
T4	Phase Feeding	Control diet	Phase Feeding	Control diet
T5	Phase Feeding			Control diet

Result and Discussion

Average feed consumption as well as body weight gain of all experimental animals from all treatments did not show significant differences. The experimental animals have similar body weight, and their weight gain was not affected by phase feeding. The birds on T5 group receiving phase feeding from 19 to 29 weeks turned out to reach earlier sexual maturity (136.85 days) compared to birds on T1, T2 and T3 groups, but did not differ from those of T4 group (table2). The effect of increasing dietary protein and energy level on sex maturity was reported by Leeson and Summers (1987) and Renema et al., (2001) on breeders type.

Table 2. Effect of phase feeding on feed consumption, age of sexual maturity, egg weight and egg production

Treatment	Feed consumption (g/day)	Sexual maturity (day)	The first egg weight (g)	Egg production (%)
T1	111.4	149.6^{b}	45.5^{b}	54.5
T2	109.6	149.5^{b}	45.0^{b}	47.2
T3	111.7	151.0^{b}	45.4^{b}	51.8
T4	109.8	145.7^{ab}	44.1^{b}	59.8
T5	108.7	136.8^{a}	41.9^{a}	55.4
probability	NS	**	*	NS

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N= 24 hens/treatment , NS= Not significant, ** = P<0.01; * = P<0.05
[a-b] In each column with different superscripts are significantly different (P<0.05)

There was no statistical effect between treatments on global egg production. Egg production at early stages of laying period, 19-20 weeks, slightly varied from 11.06 to 16.18% and some change were observed for short period. However, the application of phase feeding at several critical production periods did not considerably modify egg production.

Oviduct weight and size at the early laying period or at peak production was similar between treatments (table 3) . It increase considerable with age between 21 and 33 weeks. Feeding high protein and energy diet after sexual maturity has therefore no effect on oviduct development.

Table 3. Effect of phase feeding on length and weight of oviduct (N=5)

Treatment	Age (wk)					
	21		29		33	
	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)
T1	30.0	41.6	62.4	70.8	56.8	74.3
T2	22.2	39.0	56.8	63.6	56.6	70.8
T3	28.8	51.6	54.9	60.7	64.1	74.3
T4	23.3	50.1	65.4	71.3	61.6	76.8
T5	24.9	42.7	57.9	70.3	73.2	76.5
Probability	NS	NS	NS	NS	NS	NS

Oviduct weight is therefore affected by alternative factors as pointed out by Hocking and Robertson (2000) and Renema et al., (2001). Similarly, length of the oviduct was not affected by the treatment. However, oviduct length changed between birds at different ages after sex maturity. Yuwanta (1998) found differences on oviduct length when comparing three body weight categories layers receiving three different types of diet at 22 week of ages. The differences disappeared at 32 week of ages.

The ovary weight or follicle number was not affected by the dietary treatment (table 4) but increase with age of the hens from 21 to 33 weeks of age. Birds receiving high protein and energy diet when sex maturity was reached (T2, T4 and T5) tend to produce more ovum, but the difference did not reach significance compared with birds receiving control diet (T1 and T3) at the same age. It has been however shown that the ovary development is affected by feed intake (Yuwanta, 1998), body weight (Renema et al., 1999), light intensity (Renema et al. 2001) and bird strain or selection (Hochking and Robertson, 2000)

The number of follicles found in Leghorn type, dwarf and normal were 6.3, 9 and 12.4 (Hocking et al., 1989), and feed consumption and ages influence the follicular number as well as follicular atresia.

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Table 4. Effect of phase feeding on ovarium weight and numbre of follicle (5 hens/group)

Treatment	Age (wk)					
	21		29		33	
	Follicle weight	Follicle number	Follicle weight	Follicle number	Follicle weight	Follicle number
T1	24.0	15.0	40.7	15.0	37.6	20.0
T2	12.3	6.2	50.5	12.8	47.5	18.1
T3	21.4	10.8	39.7	16.8	45.1	23.3
T4	17.0	7.5	50.3	18.0	46.9	16.0
T5	16.7	8.3	46.4	13.8	47.3	20.8
probability	NS	NS	NS	NS	NS	NS

Egg weight was increased by the dietary treatment compare to control (table 5).

Table 5. Effect of phase feeding on egg quality, N= 50-60 eggs/treatment

Treatment	Egg weight (g)	HU	Shell Index (g/cm^2)	Yolk weight (g)
T1	51.9^a	98.3	8.1	12.4^a
T2	53.1^b	97.8	8.3	12.3^a
T3	54.0^b	98.4	8.3	12.9^b
T4	53.9^b	98.7	8.3	12.7^{ab}
T5	54.6^b	97.2	8.3	12.9^b
Significancy	**	NS	NS	**

Increasing dietary protein and energy increased the egg weight whatever the type of phase feeding, at sexual maturity of at peak of production but group 5 with a longer period of this diet showed the higher egg weight as expected. Similarly, yolk weight was increased in birds (T2 to T3) fed on diet with higher protein and energy (table 5)
Phase feeding did not affect albumen quality Haugh Unit values, but the tendency of decreasing HU in older bird was shown in the study. Shell index although showed no difference between group receiving similar mineral supply. It is known that shell quality is influenced by mineral intake and egg weight (Yuwanta and Nys, 1990), by ages of the birds, body weight and feed consumption (Yuwanta et al., 1991).

In conclusion, Introducing a high protein and energy diet at the onset of egg production (19-20 week old) and at the production peak (27-28 week old) did not affect feed consumption and egg production. It affected the weight of the first laid egg, and accelerated sex maturity completion. The oviduct and ovary weight, the numbers of follicles were not affected by the dietary treatments. In contrast, egg and yolk weight were increased in birds suppled with higher protein and energy diet.

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EFFECT OF DIETARY SUPPLEMENTATION OF ZEOLITE ON PERFORMANCE AND EGGQUALITY OF LAYING HENS

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Keywords: Zeolite,Poultry,Egg shell,Yolk,Cholesterol

Abstract

A 50-day experiment was conducted with 30-week-old laying hens to investigate the infiuence of dietary supplementation of zeolite on performance, egg production and egg quality. A total of 24000 hens were fed a control diet or one of three diets containing different levels of zeolite (20, 40 and 60 g/kg). Zeolite did not affect feed intake, egg weight, egg volume, composition of yolk, yolk color and yolk volume compared to the control. The diets with 20 or 40 g/kg zeolite increased egg prodution, egg shell thickness ($p<0.05$) and hen-day egg production ($p<0.01$). Egg yolk cholesterol did not differ among treatments. The diet with 20 or 40 g/kg zeolite resulted in a more intense egg albumen whiteness as measured by the sensory study, compared with the other diets ($p<0.05$). There was a linear relationship between dietary zeolite level and egg shell thickness.

Résumé

Une expérience a été faite pendant 50 jours sur les poules pondeuses dage de 30 semaines, pour étudier l'effet de la supplémentation alimentaire de zeolite sur la performance, la qualite et la production d'oeuf. Un nomber total de 24000 poules ont été alimentees par un regime de controle ou l'un de trois regimes dietetiques contenant des niveaux differents de zeolite (20, 40 et 60 g/kg). La zeolite n'a eu aucun effet sur la consomation d'aliment, le poids des oeufs, le volume des oeufs, la composition du jaune d'oeuf , la couleur et le volume du jaune d'oeuf en comparaison avec les controles. Les regimes contenant 20 ou 40 g/kg de zeolite ont augmenté la production d'œuf, l'epaisseur de la coquille ($P<0.05$) et production journalière des poules ($P<0.01$). La quantité de cholesterol ne diffère pas suivant le traitement. Les régimes contentant 20 ou 40 g/kg de zeolite ont intensifié la blancheur de l'albumen des œufs,

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mesurée par etudes sensorielles et comparée aux autres régimes diététiques ($P<0.05$) Une relation linéaire entre le niveau du zeolite du régime alimentaire et l'épaisseur de la coquille était observée.

Introduction

Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations, having infinite, 3-dimensional structures. The most important property of zeolites in agricultural application is the ion exchange capability(7). Clinoptilolite, [(Na4K4)(Al8Si40O96). 24H2O] one of the naturally occurring zeolites , has an ion exchange capacity for ammonium ions of 2.5meq/g. The applications of natural and synthetic zeolites for poultry production have been reviewed by Evans and farrell (1989). A total of 66 experiments were cited involving the application of zeolites to poultry production. Areas where zeolites have potential use are: a feed additive to improve poultry performance of layers and broilers, to assist in manure and litter management, and to assist in controlling air quality in poultry house environments. Nakaue et.al (1981) reported that the best method for reducing the ammonia nitrogen and litter moisture was to apply clinoptilolite on clean wood shavings at $5kg/m^2$ after 28 days of production.Nakaue and Koelliker(1981), did not found significant difference among treatments (0, 2.5 ,5 or 10% zeolites in diets) in egg weight body weight gain, shell quality or interior egg quality. They found significant effect of treatment on egg production, daily feed consumed per layer, feed conversion and fecal moisture level. In another trial, a feed mixture was supplemented with zeolite at 3% for egg type pullets, and at 5% for broiler chickens. Zeolite increased the efficiency of feed utilization for growth.
This study was undertaken to ascertain the effect of feeding varying levels of zeolite on production parameters and egg quality of layers.

Materials and methods

A total of 24000, 30-wk-old White Leghorn Hens in a farm were fed with ration containing 0, 20, 40, and 60 g/kg^{-1} zeolite for 50 day period. The hens were housed in 2 birds cages (40cm×35cm×35cm) in a naturally ventilated cages house. Each level of zeolite was fed to one line of cages (6000 birds to three replicates of 2000 hens per replicate). Water was provided in continuous V-trough-type watercress. Artificial lights were set from 05.00 to 19.00 hr when necessary to provide at least 14hr of light. Approximately 5.4-lx light intensity was allowed at bird level. The composition of the

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layer rations is listed in Table 1. All rations were fed ad libitum. The ration was formulated to be iso nitrogenous by readjusting the levels of corn when zeolite was incorporated at 20, 40 and 60 g/kg. No attempt was made to equalize the energy levels of the ration. The source of zeolites was derived from North Iran and the particle size used in this study ranged from –20 +40 mesh.

Feed intake, egg production, egg weight, feed conversion efficiency changes were recorded on a weekly basis. Egg quality parameters were measured every two weeks. The eggs were broken with a blunt knife and contents poured on a piece of flat glass positioned on a flat surface. The albumen and yolk heights were measured using a tripod micrometer. Yolk diameter was taken as the maximum cross-sectional diameter of the yolk using a pair of calipers and read on a ruler in millimeter. The yolk index was calculated as the proportion of yolk height to diameter. Hague unit scores were calculated from egg weight and albumen height using the formula $HU=100\log(H+7.57-1.7w^{0.37})$. Shell thickness was determined with a micrometer screw gauge after the removal of membranes as the average of 3 readings taken at the waist, broad and narrow regions. The component of raw eggs were determined by separating yolk from albumen using a yolk separator while shell free of membranes were oven-dried at 100^{0C} overnight and cooled to room temperature. The yolk and dried shell were weighed separately and albumen weight obtained by differences. The effect of hot water on the proportion of egg component was determined by immersing 24 eggs from each replicate in boiling water for 8 minutes. The eggs were thereafter , carefully broken out, membranes removed, albumen and yolk carefully separated and weighed as for the eggs. Egg volume and yolk volume determined by Arashmidus law. Data collected were subjected to analysis of variance and treatment means compared using the Duncan multiple range tests (Daniel 1991).

Result and discussion

The result of the performance and egg quality response of layers are presented in table 2. There was a consistent increase ($p<0.05$) in feed intake with increase in zeolites content of the diets. Layers fed 2,4 and 6% zeolite in ration had similar egg production rate, significant differences were observed only between group control and the other groups. Egg weight, egg volume and egg yolk weight were not significantly influenced by the inclusion of zeolite. Birds on 0 and 2% zeolite in diets have similar haugh unit index while those on 4 and 6% levels were also similar. The use of zeolite in layer diets positively enhanced ($p<0.05$) the shell thickness at all levels. The effect of diet and boiling on egg components of layers are shown in table 3. The inclusion of zeolite did not adversely affect the proportion of raw egg yolk and albumen. Only the values for egg shell indicated significant variations, being lower on control than the zeolite

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diets. Boiling resulted in lighter yolk, but heavier shell and membrane compared to the raw eggs. However, only the raw and boiled egg shell indicated a significant difference ($p<0.05$) among the dietary treatment while other component at both raw and boiled status showed only numerical differences ($p<0.05$).

Nakave and koelliker(1981) have observed different egg wieght, specific gravity and haugh units in the layers fed diet containing 0, 2.5, 5 and 10% clinoptilolite. These results indicate that layers fed rations containing 2% zeolite had significantly lower egg production than layer's fed rations containing 0 and 2% zeolite . however increase in daily feed consumed per layer and feed conversion were noted with increase in zeolite in the feed. The increased daily feed consumption with increasing zeolite in the feed can be attributed to the lower energy level in the feed with increasing amounts of zeolite. The observation is in agreement with reports by Altan et al (1998) and kalyuzhnkov et al (1988) .

The higher shell thickness also resulted by birds fed zeolite. Basically, diets containing 2, 4 and 6% zeolite, should consumed more elements, because of their higher mineral content in the rotiobs by used zeolites. This point was reported by Evans and Ferrell (1989). Thus, the diets were presumed to support internal egg quality, calcium metabolism and shell deposition. In this trial in terms of egg quality, haugh unitincreased with zeolite and the differences were sufficient enough to cause significant variation. The similar values obtained for shell thickness, shell weight, specific gravity and egg cholesterol across dietary treatments were close to values reported for layers in the topic (Evans and Ferrell 1989). In conclusion, the utilization of zeolite in its present form at a dietary level above 4% is of little beneficial effect to the laying hens, apparently due to the low intake at higher dietary levels. In order to improve the relative value of zeolite in layer diets, oil or molasses, can be added to mask the odor and enhance its palatability and supply energy.

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Table 1. Composition and calculated analyses of layer rations

Ingredient	**Control**	**2% zeolite**	**4% zeolite**	**6% zeolite**
Corn, yellow	69.4	67.4	65.4	63.4
Soybean meal,44% protein	19.0	19.5	20.0	21.4
Alfalfa meal	2.50	2.50	2.50	2.50
Defluorinted phosphate	1.90	1.90	1.90	1.90
Limestone flour	3.30	3.30	3.30	3.30
Oyster shell	3.30	3.30	3.30	3.30
Salt, iodized	0.25	0.25	0.25	0.25
Vitamin premix	0.20	0.20	0.20	0.20
Trace mineral premix	0.05	0.05	0.05	0.05
D,L methionine	0.10	0.10	0.10	0.10
Zeolite	0.00	2.00	4.00	6.00
Chemical analysis				
Matabolizable energy, kcal/kg	2832	2770	2708	2646
Crud protein, %	15.5	15.4	15.4	15.5
Calcium, %	3.32	3.32	3.23	3.23
Available phosphorus, %	0.45	0.45	0.45	0.45
Methionine, %	0.34	0.34	0.34	0.33
Methionine+cystine, %	0.61	0.61	0.60	0.60

Table 2. Effect of zeolite on porformance and eggquality parameters of Layers.

	Zeolite in diets (%)				
Parameters	0	2	4	6	SE
Feed intake g/d	111 [a]	115 [ab]	117 [ab]	121 [b]	2.71
Hen day egg production, %	72.6 [a]	81.5 [b]	82.1 [b]	79.4 [b]	2.71
Egg weight, g	58.1 [a]	58.3 [a]	58.6 [a]	59.0 [a]	0.61
Egg volum. Ml	56.4 [a]	55.5 [a]	54.8 [a]	55.8 [a]	0.58
Havgh unit, %	82.1 [a]	82.9 [a]	84.8 [b]	83.4 [ab]	0.82
Shell thickhess,mm	0.33 [a]	0.38 [b]	0.36 [bc]	0.38 [c]	0.007
Egg yolk weight, g	17.3 [a]	17.2 [a]	17.2 [a]	17.5 [a]	0.008
Egg,yolk choles terol, mg	190 [a]	182 [ab]	180 [b]	175 [b]	1.58
Shellweight. g	6.01 [a]	6.14 [b]	6.51 [ab]	6.71 [c]	0.22
Specific gravity	1.03 [a]	1.06 [b]	1.07 [b]	1.06 [b]	0.002

means with different supescripts on same row are significantly different (p<0/05).

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Table 3. Effect of zeolite on raw, boiled and average valuo of egg components

	Zeolite in diets (%)				
Components	0	2	4	6	SEM
Yolk					
raw	29.77	29.50	29.35	29.60	0.21
boiled	27.12	27.09	27.40	27.35	0.35
Albumen					
raw	57.71	58.10	58.13	58.09	0.41
boiled	58.35	59.27	59.30	59.12	0.89
Shell					
raw	10.05 [a]	10.28 [b]	10.47 [bc]	10.65 [c]	0.18
boiled	10.34	10.37	11.11	11.37	0.31
Membrane					
raw	1.51	1.52	1.55	1.53	0.08
boiled	1.75	1.76	1.72	1.78	0.12

means with different supescripts on same row are significantly different (p<0/05).

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LUTEIN DERIVED FROM MARIGOLD MEAL, STANDARDIZED BUT NOT SOLVENT EXTRACTED, IS READILY DEPOSITED INTO EGG YOLKS

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Keywords : egg yolk, lutein, natural pigmenter, Organic

Abstract

The use of natural pigmenter products based on solvent-extracted marigold meal for egg yolk pigmentation is well established and very successful. However EU regulations concerning "Organic" egg production do not permit the use of solvent-extracted feed ingredients. The Kem Gold dry product is a pigmenter based on marigold flowers from fully integrated production under strictly controlled growing and processing conditions, including full traceability. This completely natural and not solvent extracted standardized marigold meal with a small, uniform particle size, containing 13.5 g/kg total yellow carotenoids was investigated as a pigmenter suitable for Organic egg production. The trial was conducted on three separate commercial layer farms. Standardized marigold meal was incorporated into the commercial layer feeds at 6.4 kg/ton of feed. A total of 435 eggs were collected from the three farms and individually assessed for yolk colour using the Roche colour fan. The lutein content in the yolk of these eggs was also determined. A visually attractive yolk colour with a mean colour fan value ranging between 9-11 was always obtained. The eggs contained a mean lutein content of 1.32 ± 0.47 mg lutein / egg. Clearly standardized marigold meal is a very suitable source of lutein for pigmentation of eggs produced under Organic farming.

Résumé

L'usage de pigments naturels, à base d'un extrait de farine du *Tagetes erecta*, destinés à la pigmentation du jaune d'œuf est bien établi. Pourtant, les règlements UE concernant la production biologique d'œufs n'autorisent pas, pour les aliments, l'usage d'ingrédients issus d'une extraction par solvant . C'est pourquoi une farine de *Tagetes erecta* de granulométrie fine et uniforme, contenant 13,5 g/kg de caroténoïdes jaunes a été évaluée pour la coloration d'œufs biologiques. L'essai a été conduit sur trois

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fermes. La farine du *Tagetes erecta* a été incorporée aux aliments pour pondeuses à une dose de 6,4 kg/tonne d'aliment. Un total de 435 œufs provenant des trois fermes a été rassemblé et leurs jaunes évalués individuellement, en utilisant l'index de couleur de Roche. La teneur en lutéine de ces œufs a également été déterminée. Une couleur de jaune d'œuf visiblement attrayante avec un index moyen de couleur situé entre 9 et 11 a toujours été obtenue. Les œufs contenaient une teneur moyenne en lutéine de 1,32 ± 0,47 mg lutéine/ œuf. La farine du *Tagetes erecta* standardisée est donc une source de lutéine très convenable pour la pigmentation d'œufs produits biologiquement.

Introduction

The use of natural pigmenter products for egg yolk colouring is well established and the interest to colour egg yolk is still increasing. In poultry, β-carotene is almost completely converted into vitamin A or is otherwise metabolized. As a consequence β-carotene does not contribute to the pigmentation of poultry eggs. The pigmentation of poultry products is based on the absorption and deposition of oxycarotenoids such as lutein and zeaxanthin (Hencken, 1992). The Kem Gold product is a pigmenter based on marigold flowers from fully integrated production under strictly controlled growing and processing conditions, including full traceability. It is a perfect source of lutein frequently used to colour egg yolk. It is completely natural and is not solvent extracted. Consequently it is suitable for use in all poultry feeds, including organic and bio-feeding. This study demonstrates the efficiency of Kem Gold in pigmenting egg yolk and discusses pigmenting data obtained from industrial layer farms.

Material and Methods

This trial was carried out in cooperation with three layer farms. Layers were fed a commercial feed supplemented with 6.4 kg Kem Gold / ton of feed containing 13.5 g / kg yellow carotenoids. Eggs were randomly collected from those three farms and were analysed for their lutein content. The study was carried out from February till June 2002. Yolk colour was assessed by the Roche colour fan. Analysis of the lutein content was carried out according to the AOAC method (AOAC, 1990). Lutein content was expressed as mg lutein / kg yolk and as mg lutein / egg. A considerable number of eggs were analysed, which ranged between 120-165 eggs for each farm. In total, more than 430 eggs were individually analysed for their lutein content. This large data pool allows a detailed statistical analysis of the results.

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Data were statistically analyzed by Microsoft Excel Statpro. Values reported are the mean, standard deviation, minimum, maximum, median, first and third quartile and interquartile range and have the following interpretation. The minimum is the lowest value, the maximum is the highest value reported in the data set. The median is the middle observation when the data are listed from smallest to largest value. The median splits the data in two halves and is similar to the 50th percentile because half of the data are below the median and half of the data are above the median. The first quartile means 25% of the data have a value below this number and 75% have a value above this value. The third quartile value indicates 25% of the data have a value higher than this number. The interquartile range covers the range between the first and the third quartile. Significance difference between the farms was checked by One-Way ANOVA and the Tukey test.

Results and Discussion

Addition of the natural pigmenter Kem Gold to the feed significantly improved the yolk colour and a considerable lutein deposition in the egg was found. Mean and median of the yolk colour ranged between 9-11 over the 3 farms and the standard deviation was only 1 unit across all farms. This standard deviation is acceptable and is crucial for a correct interpretation of the results. The inter quartile range showed that in farm 1, 50% of the eggs were between 10 - 11, in farm 2 between 9 - 10 and in farm 3 between 10 – 12 in yolk colour. This indicates good homogeneity in egg yolk colouring.

Within the three farms little differences in egg yolk colouring and lutein deposition was observed. Small differences in pigmentation could be explained by external factors, including differences in feed, layer genotypes, environmental factors etc. Data of the three farms were combined and interpreted together, giving the best approximation of the situation encountered in industrial practice.

The overall results obtained from 435 analyses are listed in Table 1. The mean yolk colour obtained from layers supplemented with 6.4 kg Kem Gold / ton was 10±1. The first quartile shows a yolk colour of 9, which indicates that in 75% of the analyses the yolk colour will at least have a value of 9 or even higher. For 25% of the eggs, the yolk colour will be higher than 11. As a consequence 50% of the eggs will have a yolk colour varying between 9-11. In order to obtain a consistently darker yolk colour it would be necessary to introduce a red pigmenter in the feed (Nys, 2000).

A mean lutein content of 1.32 ± 0.47 mg lutein / egg was observed. In 75% of the eggs at least 1.02 mg lutein / egg was present. At least 25% of the eggs had a lutein content higher than 1.46 mg lutein / egg. Eggs analysed from layers not supplemented with pigmenters have a significant lighter yolk colour and a minor lutein content. The mean

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lutein content of eggs not supplemented with pigmenters analysed in the past in our laboratory is 0.16 mg lutein / egg. Supplementation of the feed with 6.4 kg Kem Gold / ton feed always resulted in a mean lutein content above 1.1 mg lutein / egg.

Table 1. Data on the Kem Gold pigmentation of industrial layer eggs (435 eggs analysed)

Parameter	Roche Yolk colour	mg lutein / kg yolk	mg lutein in egg
Mean	10	85	1.32
Median	10	77	1.22
Standard deviation	1	30	0.47
Minimum	6	39	0.61
Maximum	13	220	3.14
First quartile	9	65	1.02
Third quartile	11	93	1.46
Interquartile range	2	28	0.44

The pigmenting efficiency depends on several factors, including the digestibility, transfer, metabolism and deposition of the pigment in the target tissue (Hencken, 1992). The results presented here are quite solid as they are based on a large number of eggs analysed from three commercial farms. Supplementation of layer feeds with 6.4 kg Kem Gold / ton of feedas natural pigmenter gave a significant deposition of lutein into the egg yolk. This study indicates that Kem Gold is a very efficient pigmenter for egg yolk colouring. As Kem Gold is a natural pigmenter obtained without solvent extraction it is compatible with organic and bio-feed regulations as an effective egg yolk pigmenter.

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XANTHOPHYLL PROFILE OF CORN GLUTEN MEAL THROUGHOUT THE YEAR AND TRANSFER YIELD INTO EGG YOLK

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Keywords : corn gluten, xanthophylls, lutein, egg yolk

Abstract

In the current study an HPLC method has been settled to analyse the changes in xanthophyll composition of corn gluten due to the process of production, duration of storage and to study the yield of transfer to yolk. Lutein (42-45%) and zeaxanthin (20-30%) are the predominant carotenoids in corn gluten. Gluten xanthophylls level is the highest after maize harvest until January then declines slowly to a minimum in June. 85% of xanthophylls are extracted from maize during the process of corn gluten production. The yield of transfer to yolk of xanthophylls is the highest for lutein and zeaxanthin (25 to 30 %) but is slightly lower for cryptoxanthin (14-17%). These data confirm the interest of corn gluten for egg yolk pigmentation.

Résumé

Une méthode par HPLC a été développée pour analyser les variations en caroténoïdes du gluten de maïs au cours de son processus de fabrication, en fonction du mois de l'année et pour déterminer le rendement de transfert des différents caroténoïdes dans le jaune d'œuf. Les pigments prédominants sont la lutéine (42-45%) et la zéaxanthine (20-30%). La teneur en xanthophylles totaux du gluten est maximale juste après la récolte du maïs et jusqu'en janvier, puis décroît légèrement jusqu'en juin. 85% des xanthophylles sont accumulés dans le gluten lors du processus d'extraction du maïs. Le transfert de lutéine et de zéaxanthine dans le jaune est le plus élevé (25 à 30 %). Il est plus faible pour la cryptoxanthine (14-17%). Ces données confirment l'intérêt du gluten comme source de pigment pour colorer le jaune d'œuf.

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Introduction

Colour identifies the product and evokes the taste and smell which are expected by the consumer. This is the case for egg yolk and consumers expressed preferences in yolk colour depending on their geographical area. Since hens cannot synthesize carotenoids, the uniform intense colour of the yolk is directly dependant on the dietary supply (Nys, 2000). Various sources of pigment are available from plants (corn, alfalfa, marigold concentrate, and paprika) and synthetic origin (canthaxanthin, apo-carotene ester, citranaxanthin). Their efficiency depends on pigment digestibility, metabolism and rate of deposition but also on their stability. One of the most frequent sources of natural pigment is yellow corn either whole or in the form of a derivative: corn gluten. The colorimetric method commonly used (method A - ISO 6558) quantifies total xanthophylls but does not differentiate the components. Only HPLC method allows the identification and quantification of the components (Hamilton, 1992). Our objective was first to optimise an HPLC method for analysing corn gluten xanthophylls, to evaluate the origins of change in corn gluten oxycarotenoid composition (maize origin, duration of storage, process of gluten production) and to estimate the yield of the various xanthophylls transfer to the yolk.

Materials and methods

Sample collection: The corn gluten was manufactured by Roquette **(**trademark GLUTALYS®). Two batches of corn and of corn gluten were placed under different storage conditions (4°C and 20°C; dark and light) over a 5-month period for studying the stability of product.

Sample preparation: samples were ground, introduced and maintained in an ethanolic solution of potassium hydroxide for 30 minutes under nitrogen flow. The pigments were extracted using ethyl ether. The ether phase was washed with fractions of potassium hydroxide solution and water, dehydrated on sodium sulphate then evaporated. The extract was taken up in petroleum ether for colorimetric analysis and in methyl tertiary-butyl ether (MTBE) for chromatographic analysis. The solution of carotenoids in petroleum ether was separated into two fractions containing the carotenes or xanthophylls using an aluminium oxide column (OD = 450 nm for global quantification).

The solution of carotenoids in MTBE was injected into a chromatographic system consisting of a 5 µm polymeric C30 silica column (250 X 4.6 mm, porosity 200 Å), a gradient pump (Varian 9012) and a spectrophotometric detector (WATERS 486) set at 450 nm. The mobile phase consisted of a mixture of polar solvents A (MTBE-methanol-

water: 15-81-4) and apolar solvents B (MTBE-methanol-water: 90-6-4) applied in a linear gradient (T0 = 100%A; T90min = 100%B; elution: 1 ml/min; temperature: 25°C). Calibration was performed using standard solutions of lutein and β-carotene (FLUKA) and of zeaxanthin, β-cryptoxanthin and canthaxanthin (ExtraSynthèse) prepared in MTBE.

Xanthophylls transfer to yolk: the trial was performed on three groups of 10 Hubbard-Isa hens for 6 weeks. The three experimental diets contained 8 % of white corn gluten (26 mg xanthophylls / kg), 8 % of yellow corn gluten (350 xanthophylls mg/kg) and 4 % of each corn gluten sources. They were formulated according to the recommendation of INRA (70 % wheat, 8 % soybean, 2.5 % vegetal oil; 11.900 KJ, 16.8 % Protein, 3.6 % Ca). Eggs were collected for analysis 5 and 6 weeks after introduction of the experimental diets. Yolks of 3 eggs laid by the same hens were pooled for analysis.

Results and discussion

Numerous chromatographic methods have been proposed for analysing carotenoids in plants (Livingston et al., 1968; Hamilton, 1992). The current HPLC method demonstrates a good separation of the various components (figure 1a) and satisfactory intra-laboratory repeatability (CVr <3% for corn, <10% for gluten carotenoids). The total xanthophyll level was, however, higher when quantification was carried out using the HPLC method compared with the colorimetric method (+10 to 14 % for corn gluten and + 20 to 30 % for corn).

The concentration of total xanthophylls in corn gluten meals observed in table 1 is in agreement with previous observation for a high protein gluten corn meal (El Boushy and Raterink, 1989: 330 mg/kg) with however an higher mean concentration compared with older data (361 mg/kg in lieu of 260 mg/kg, Marusich and Bauernfeind, 1981; Nys, 2000). That is likely to reflect the improvements in xanthophylls extraction and in storage conditions.

Table 1: oxycarotenoid content of corn gluten (protein: 59.2% ; n=28) and corn (n=11).

	Corn Gluten (mg/kg)		Corn
	Mean	range	(mg/kg)
Total xanthophylls	361 ± 46	278-440	31 ± 4
lutein	155 ± 19	117-205	15 ± 2
zeaxanthin	91 ± 18	60-161	9 ± 2
β-cryptoxanthin	14 ± 5	7-35	1 ± 1
other pigments	84 ± 7	71-99	5 ± 1
β-carotene	7 ± 2	3-12	1 ± 0
other carotenes	9 ± 2	6-13	<1

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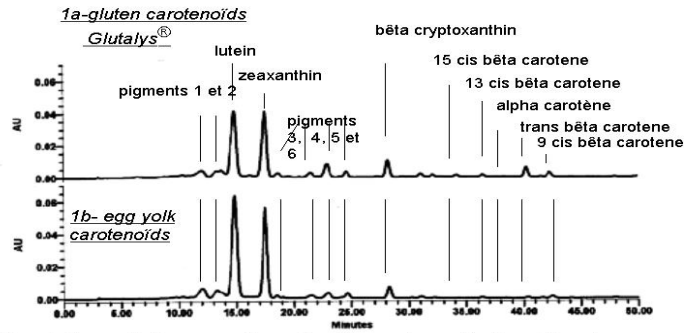
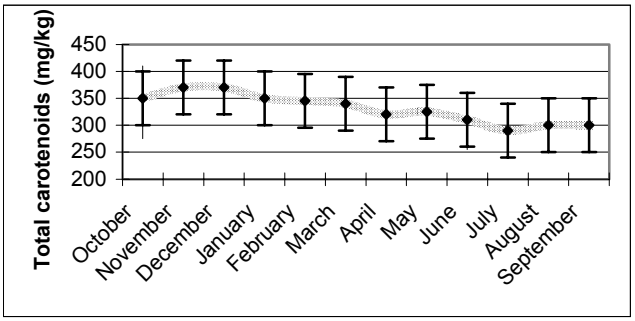


Figure 1: Carotenoid chromatographic profile of gluten and egg yolk of hens fed on gluten

Lutein (42-45%) and zeaxanthin (20-30%) are the predominant components of corn gluten in agreement with others (Livingston et al., 1968. There are present as free form and are efficiently absorbed by the intestine (Hamilton, 1992; Hencken 1992). Other pigments (table 2) are isomers from lutein and zeaxanthin and are efficiently transferred in yolk. β-carotene and other carotenes may represent 10% of total carotenoids but do not contribute to yolk coloration in poultry.

The change over the year in the total carotenoid content of corn gluten (GLUTALYS®) was determined in 6 campaigns, from 1994 to 1999, by the colorimetric method, to assess the effect of corn storage duration (figure 2).

Figure 2: change in total carotenoids in corn gluten over the year (monthly means from 1994 to 1999, n=290).



The highest contents were observed at the start of the campaign until January. Then, xanthophylls level slightly decreased to stabilize in august to September. The corn gluten composition reflects that of the batch of maize used to produce the gluten. That was confirmed by tracing the composition of the derivatives products throughout the steps of production (dry corn, steeped corn, full milk, protein cake, corn gluten, fibres).

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The follow-up shows that 85 % of corn pigments are concentrated in gluten. Fibres contain 8% of pigments. Other corn derivatives contain negligible quantities of pigment. Finally, the study of the stability of gluten (GLUTALYS®) shows that the pigment content remains unchanged in corn gluten stored for two months at 4°C or 20°C (data not shown).

Corn gluten meal xanthophylls transfer yield in egg yolk: early literature indicates that the efficiency of corn gluten for yolk coloration was slightly lower than that of the corresponding maize (88%, range from 80 to 96 % , Marusich and Bauernfeind, 1981). This observation is in agreement with the fact that the yield of pigment extraction in the factory corresponded to 85%.

Table 2: concentration of xanthophylls in laying hen diets (mg/kg), egg yolk (mg/kg) and yield (%) of transfer to the yolk.

Level of corn gluten	White Corn Gluten (8%)			Yellow (4%) and White (4%) gluten			Yellow corn gluten (8%)		
Yolk colour*	5.9 ± 0.3			8.8 ± 0.3			10.5 ± 0.56		
	Diet	Yolk	Yield	Diet	yolk	yield	diet	yolk	yield
Total xanthophylls	5.45	8.5	22.7	17.3	27.4	25.8	28.5	46.8	25.1
Lutein	3.25	5.6	24.8	8.05	14.9	29.9	13.3	24.3	27.8
Zeaxanthin	1.1	1.4	18.8	4.9	7.6	25.1	7.95	13.5	25.7
β-cryptoxanthin	0.1	0.1	14.4	0.85	0.7	13.3	1.3	1.5	17.4
Other pigments	1	1.4	21.9	3.5	4.3	20.3	5.95	7.6	19.8
Total carotenes	<0.1	<0.1	ND	1	1.2	6	1.4	1	5

* Roche index was calculated using the Cielab system (L*, a*, b*; n=60) (Nys, 2000).

There are also numerous experimental data showing that corn gluten meal efficiently contributes to yolk coloration. Its colouration capacity is slightly higher than that of alfalfa (90% lutein) because of the presence of a higher level of zeaxanthin and cryptoxanthin which are more reddish than the yellow lutein.

The yellow corn gluten meal contains 350 mg xanthophylls /kg and 363 mg total carotenoids /kg. The corresponding profile of gluten and egg yolk is shown in figure 1. Lutein, zeaxanthin, cryptoxanthin and other pigments show associated peaks in both chromatographic profiles demonstrating a transfer from corn gluten to yolk. In contrast, the carotene peaks were reduced in egg yolk extracts compared with their dietary source.

The calculation of the yield (table 2) confirms this observation as it was around 5% for carotene and of 15 to 30% for xanthophylls. The yield was calculated taking into account the measured level of pigments in diet and egg yolk, the feed consumption and the egg production during the period of egg collection. The accumulation of xanthophylls in yolk increases in proportion to the dietary xanthophylls. The yield of transfer of xanthophylls was 25% and was not affected by the level of dietary xanthophylls. The yield is higher for lutein and zeaxanthin but is lower for β-

cryptoxanthin (table 2). The yolk colour increases with the dietary level of xanthophylls but the increase is not proportional as yolk coloration tends to plateau at high level of dietary supply (Nys, 2000).

In conclusion, this study confirms that corn gluten is a reliable and predictable source of carotenoids in poultry feed. Corn gluten is stable during a few months of storage. The annual change is identical from year to year and therefore predictable. Transfer to the egg is effective and remains stable at various dietary supplies. Lutein, zeaxanthin, and β-cryptoxanthin, the predominant oxycarotenoids of corn gluten, are efficiently transferred to the yolk. Corn gluten is therefore a useful source of pigment for yolk coloration. In addition, corn gluten contains a high proportion of lutein, opening the prospect of its value-enhancement in human for prevention of certain pathology associated with age such the macular degeneration of the eye and of enrichment of the human diet in pigments of natural origin with anti-oxidant properties.

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EGG QUALITY TRAITS UNDER HEAT STRESS WITH OR WITHOUT SUPPLEMENTAL YEAST

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Keywords : albumen quality, shell quality, heat stress, yeast

Abstract

External and internal quality traits were studied in eggs laid by White Leghorn hens subjected to heat stress with or without a yeast supplement added to their diet. The analysed traits were thick albumen height, egg specific gravity, and shell weight. Significant differences among treatments were observed for egg specific gravity and percentage shell in both experimental groups irrespective of whether they had received supplemental yeast or not. It was concluded that heat stress significantly decreases egg quality and that dietary yeast supplementation had no beneficiary effects on eggshell quality under these conditions.

Résumé

Des critères de qualité interne et externe de l'oeuf ont été étudiés sur des œufs de poules Leghorn blanches soumises à des stress thermiques et supplémenté ou non avec de la levure. Les caractères analysés étaient la hauteur d'albumen épais, la gravité spécifique de l'œuf et le poids de coquille. Des différences significatives entre les traitements sont observées pour la gravité spécifique de l'œuf et le pourcentage de coquille dans les 2 groupes d'œufs issus des poules ayant eu un stress thermique en présence ou non d'une supplémentation enlevure. Le stress thermique diminue doonc significativement la qualité de coquille sans que cet effet négatif soit corrigé par une supplémentation en levure

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Introduction

Studies on the utilization of yeast as a source of protein have indicated its suitability for starter chicks (Woodham and Deans, 1973). Besides, the use of yeast has been reported to improve the availability of phytate phosphorus (Thayer and Jackson, 1975).

The effects of yeast on the production and quality performances of poultry have mainly been studied under normal environmental temperature. For example, Yoshida et al. (1974) and Yoshida (1975) found no difference in Haugh units or shell thickness for eggs laid by hens fed with 15% yeast with respect to control hens. Thayer et al. (1978) reported an improvement in egg production, egg weight and hatchability for turkey hens fed diets containing 2.5% live yeast culture. Results obtained by Day et al. (1987) indicated a non beneficial effect of yeast on egg production, feed efficiency, egg weight, and shell strength, when fed at 0.25 or 0.50% of the diet. Sunder et al. (1990) reported that egg weight, Haugh units, egg specific gravity, shell density, and shell weight were similar in dietary groups containing 0, 10 or 15% inactive dry yeast, but layers fed 15% yeast had higher shell thickness, and shell weight per unit of surface area. In Broiler chickens, Miazzo et al. (2000), Nadgauda et al. (2000), and Santin et al. (2000) found that supplemental yeast at 0.5-8%, 0.15%, and 0.2%, significantly improved weight gain and feed efficiency.

Yeast is also known to reduce stress in animals (Phillips and Von Tungeln, 1984). The use of yeast at 0.8% reduced significantly the effects of aflatoxicosis (Stanley et al., 1993), and its use at 10% reduced the frequency of *Salmonella* and *Campylobacter* colonization in broiler chickens subjected to transport stress (Line et al., 1997). Chukwu and Stanley (1997) found that the production performance of laying hens during severe high temperature could be maintained with dietary inclusion of yeast at 0.5%. Guo and Liu (1997) reported that supplemental yeast in the feed significantly decreased the heterophil to lymphocyte ratio (an indicator of the stress response in chickens) in broilers under heat stress, even though Campo and Dávila (2002) did not find significant effect of dietary brewer`s yeast (*Saccharomyces cerevisiae*) supplementation at 0.5% on stressfulness of White Leghorn hens.

It is a well-known fact that eggshells become more fragile when hens are subjected to high temperatures. High temperatures decreased both shell quality and egg production in the studies reported by Campos et al. (1960), Bragg et al. (1971), Arima et al. (1976), Wolfenson et al (1979), and Grizzle et al. (1992). Huston (1958) found a significant reduction in egg weight and egg specific gravity at high environmental temperature, although albumen quality was not affected.

The objective of this work was to evaluate the effects of supplemental yeast in the diet on the external and internal quality of eggs laid by White Leghorn hens under heat stress.

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Materials and methods

The data were obtained from 40-wk-old hens from a White Leghorn population, originated from crossing three strains selected for egg number and egg weight (Campo and Jurado, 1982). Two treatment groups were used. Group 1 consisted of 40 hens subjected to 37.5ºC heat stress for three days. Group 2 consisted of 40 additional hens subjected to heat stress (37.5ºC for three days) with brewer's yeast supplemented at 0.5% in diet. There was an additional group of 40 hens without heat stress (control). Hens in each treatment were kept in cages with four hens per cage. They were fed standard layer diet (containing 16% CP, 2.700 kcal ME/kg, 3.5% Ca, and 0.5% available P). Feed and water were provided ad libitum. The room temperature of the hens prior to heat stress was about 25ºC.

The analysed traits were thick albumen height, shell weight, and egg specific gravity. Thick albumen height was determined, halfway from the yolk to the edge, to the nearest 0.01 mm using a tripod micrometer. The Haugh unit score (Haugh, 1937) was determined next by the formula modified by Eisen et al. (1962): 100 log (H – 1.7 $W^{0.37}$ + 7.57), where H is albumen height (mm) and W is egg weight (g). Egg weight and shell weight were measured to the nearest 0.1 g. Egg specific gravity was measured by the flotation method (Wells, 1968), using nine solutions of sodium chloride ascending from 1.068 to 1.100 g/cm^3 in increments of 0.004 g/cm^3, recording the solution in which an egg just floated.

A completely randomised design was used. The differences among treatments were tested with a one-way analysis of variance. The statistical model was: $x_{ij} = \mu + T_i + \varepsilon_{ij}$, where x_{ij} is the analysed measurement, μ is the overall mean, T_i is the effect of treatment (i = 1…3), and ε_{ij} is the residual (j = 1…40). Significant differences between treatments were determined using the Student-Newman-Keuls' multiple range test.

Results and discussion

The results of the experiment, indicating yeast effects on external and internal egg quality are shown in Table 1. Thick albumen height corrected (Haugh units) or not for egg weight, did not differ significantly among treatments. This finding agrees with that of Huston (1958), who reported that albumen quality of the eggs was not affected by high environmental temperature. Shell weight did not differ significantly among treatments. There were significant differences ($P < 0.01$) among treatments for percentage shell and egg specific gravity such that the mean values for heat-stressed hens and heat-stressed hens with supplemental brewer`s yeast were consistently less than that those of the control hens. These results are in agreement with those reported

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by Campos et al. (1960), Bragg et al. (1971), Arima et al. (1976), Wolfenson et al (1979), and Grizzle et al. (1992), indicating a reduction in shell quality of eggs laid by hens under heat stress. They also agree with the findings of Yoshida et al., 1974; Yoshida, 1975; Day et al., 1987; and Sunder et al., 1990 who failed to find any benefit of yeast supplementation on shell quality under normal environmental temperatures. The production performance of laying hens during heat stress can be maintained with dietary inclusion of yeast at 0.5% (Chukwu and Stanley, 1997), but this effect was out with the scope of the current study.

Table 1. Mean values and residual mean squares for quality traits in three groups of hens differing in heat stress and supplemental yeast

Trait	Heat stress	Heat stress + yeast	Control	Residual mean square
Albumen height[1]	5.63[a]	5.94[a]	5.73[a]	1.08
Haugh units	73.44[a]	75.84[a]	74.81[a]	56.80
Egg weight (g)	58.00[a]	59.12[a]	57.32[a]	22.94
Shell weight (g)	6.82[a]	7.04[a]	7.23[a]	0.72
Percentage shell	11.75[b]	11.91[b]	12.62[a]	1.07
Specific gravity[2]	96.46[b]	97.41[b]	101.47[a]	36.90

[a,b] Mean values within the same trait with no common superscript differ significantly ($P < 0.01$), [1] mm; [2] $(g/cm^3 - 1) \times 1000$

It is concluded that heat stress significantly decreased eggshell quality (percentage shell and egg specific gravity) and that brewer`s yeast supplementation did not offset this negative effect.

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BENEFITS OF ORGANIC MINERALS IN COMMERCIAL LAYING HEN PERFORMANCE AND EGG QUALITY

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Keywords: laying hens, Eggshell 49, organic minerals, eggs

Abstract

The replacement of standard minerals with organic minerals in laying hen diets was examined in a 12 week trial. Six replicates of ten laying hens received either a negative control diet or one supplemented with additional organic zinc and manganese (Eggshell 49, Alltech Inc.). Hen performance, egg production and quality were measured weekly. Results showed that feeding organic minerals improved egg weights and eggs per hen day by 1%, reduced cracked eggs by 31%, gave 6% fewer dirty eggs and 2% higher egg mass. Feed intake of the trial diet increased by on average 2g per hen per day. Shells were 1.5% thicker with the organic minerals at the end of the trial period, and detailed investigations suggested this was due to increased mineralisation.

Résumé

Le remplacement des minéraux standards par des minéraux organiques dans les aliments pour pondeuses formulés suivant les normes NRC a été etudié au cours d'un essai d'une durée de 12 semaines sur des pondeuses.
Six repetitions de 12 poules pondeuses ont reçu un régime témoin ou un régime supplementé avec du zinc et du manganèse organiques (Eggshell 49, Alltech. Inc.).
Les performances des pondeuses, la production et la qualité des oeufs ont été mesurées chaque semaine.
Les résultats ont montré que la supplémentation des minéraux organiques dans l'alimentation a amélioré le poids des oeufs et la production quotidienne d'oeufs de chaque poule de 1%, a réduit le nombre d'oeufs fêlés de 31%, a donné 6% de moins d'oeufs sales et le poids des œufs a été augmenté de 2%.

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La consommation d'aliments dans le régime essai a augmenté de 2 g en moyenne par poule et par jour. Les coquilles étaient plus épaisses de 1,5% avec les minéraux organiques à la fin de la période d'essai, expliqué certainement par l'augmentation de minéralisation.

Introduction

The mineral nutrition of laying hens is of significant importance due to the animals reliance on key elements to maintain its own physiological structures as well as to produce eggs with sufficient shell thickness to be saleable. Bell (1998) estimated that egg breakage could cost $1 per hen per year, amounting to significant economic losses. Although it is generally recognised that mineral nutrition of the hen is important, limited research into improving mineral supply is available, as most has focussed on the amounts of minerals supplied, rather than the form used. Minerals such as Manganese and Zinc are important in shell production as they are key components of enzymes required to catalyse carbonate and mucopolysaccharides in the shell membrane (Gomez-Basuri, 1998). The form of dietary minerals has been found to be of crucial importance in animal nutrition. Inorganic minerals do not have the same bioavailability as minerals chelated to amino acids ('organic' minerals) (Whitehead et al, 1996). This is due to the organic minerals ability to be absorbed and utilised in the same way as the amino acid chelated to them, which is more efficient as it is the same delivery system as mineral structures found in plant materials (Ashmead, 1993 ; Power & Horgan, 2000). Improved uptake and utilisation of minerals also has consequences for reducing pollution from egg production facilities (Miles 1998). Previous research conducted in the USA using an organic manganese and zinc combination product, Eggshell 49®, has shown positive benefits on shell quality in laying hens from 20 to 60 weeks old (Scheideler & Ceylan, 1999). Eggshell 49® has been shown to be particularly effective in maintaining shell quality in the later periods of lay (Klecker & Zeman, 1996), and the aim of the following experiment was to investigate the effects of Eggshell 49® on laying hen performance and egg quality parameters in the last 12 weeks of production under commercial conditions in Europe.

Material and Methods

Wheat soy diets formulated to meet the nutritional requirements of birds at the end of lay (NRC standards) were fed to layers housed at a commercial site in the UK. The control diet contained dicalcium phosphate (DCP) and phytase. The experimental diet

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contained Eggshell 49® (Alltech Inc.) at a level of 1 kg/t inclusion in the feed. Each diet was fed to 6 replicates of 10 birds for a period of 12 weeks at the end of the laying cycle (48-60 weeks). Standard production data including daily feed intake, egg numbers (% hen day), FCR, egg weights, egg mass, % cracks, % dirty eggs, shell thickness and shell density was measured on a weekly basis and then combined to give an average performance for the whole experimental period. In addition 12 eggs per treatment were collected at the start, middle and end of the experiment and analysed for physical measurements including egg weight, length, breadth, shape index, breaking strength, stiffness, effective shell thickness, Eshell (Youngs modulus) and electron microscopy to examine shell structural variations.

Results and discussion

Statistical analysis of treatment effect for the main parameters measured was not possible, due to the commercial nature of the trial. However there were some interesting numeric differences of commercial relevance. Performance data over the whole 12 week period showed that hens receiving Eggshell 49® improved egg weight and eggs per hen day by 1% compared to a control of 67g and 84.5% respectively. Overall birds fed Eggshell 49® had 31% fewer cracked eggs than the control, gave 6% less dirty eggs and 2% higher egg mass compared to the control 56.6g. Although there was no difference in FCR, feed intake increased by 2 g per hen per day with Eggshell 49®. Analysis conducted with specialised ultrasound methods showed that the eggs fed the experimental birds has a higher degree of mineralisation and more uniform shell structure than the control. The shells of eggs from the experimentally fed hens were 1.5% thicker at the end of the trial than those from the control. These findings would contribute to eggshell strength and fewer broken eggs.

Table 1 : Egg production data from 48-60 week old hens fed organic or inorganic mineral sources

Parameter	Control	Eggshell 49® 1kg/t
Egg weight (g)	67.0	67.8
Egg Mass (g)	56.6	57.9
Dirty eggs (%)	2.69	2.53
Cracked eggs (%)	4.04	3.08
Egg/hen/day	84.5	85.5
Daily feed intake (g)	116	118
FCR	2.05	2.03

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Table 2 : Shell characteristics of eggs from hens fed organic or inorganic mineral sources

Parameter		Control	Eggshell 49® 1kg/t
Shell thickness (mm) :			
	48 weeks	0.350	0.351
	54 weeks	0.348	0.349
	60 weeks	0.336	0.341
Egg density :			
	48 weeks	81.3	81.7
	54 weeks	79.2	81.2
	60 weeks	78.4	79.0
Total Score* :			
	48 weeks	32.7	29.7
	54 weeks	34.0	29.3
	60 weeks	32.9	30.4
Eshell :**			
	48 weeks	35049	35220
	54 weeks	34711	35034
	60 weeks	36507	38092

*Total Ultrasound score indicates variability of shell structure
** Eshell score indicates level of mineralisation in shell

Conclusions

Although it is more difficult to detect significant effects between treatments within a commercial trial environment, nevertheless hens fed Eggshell 49® showed strong numeric trends in increased shell quality characteristics and production, equating to better performance and more saleable eggs. The egg performance improvements observed in other research trials (Sheideler & Ceylan, 1999) can also be observed under commercial conditions, demonstrating that supplementing hen diets with organic minerals can improve egg quality and production.

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QUANTIFICATION OF EGGSHELL MATRIX PROTEINS BEFORE AND AFTER MOULTING

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Keywords: hen, eggshell, matrix, molt

Abstract

Molt was used to study any relationship between mechanical properties of eggshell and its organic matrix concentration. It was induced by zinc oxide (20g zinc/kg diet) in 59 ISA-Brown laying hens at 78-week of age. No significant differences were observed for egg or shell weights. On the other hand, molt improved the eggshell breaking strength (28.09 and 33.71 N before and after molting,). No modification was observed for eggshell crystal orientation but crystal size was reduced after molt. The total proteins in eggshell were slightly increased after molting (18.26 and 20.65 mg/g shell). On the contrary, the concentration in ovotransferrin was sharply decreased (11.69 and 5.54 mg/g eggshell before and after molting). These observations suggest that changes in organic matrix level and that in eggshell crystal size may be involved in the improvement in eggshell solidity after molting.

Résumé

La mue a été provoquée pour analyser la relation entre les propriétés mécaniques de la coquille et la concentration de sa trame organique. Elle a été induite par de l'oxyde de zinc (20g zinc/kg d'aliment) chez 59 poules ISA Brown âgées de 78 semaines. Aucun effet significatif de la mue n'a été observé sur le poids des œufs et des coquilles. La mue a amélioré la charge à la rupture des coquilles (28,09 et 33,71 N avant et après la mue). L'orientation des cristaux de coquilles n'est pas modifiée mais leurs tailles sont diminuées après la mue. La quantité de protéines totales dans les extraits de coquilles a été légèrement augmentée après la mue (18,26 avant la mue et 20,65 mg/g coquille après). A l'opposé, la concentration

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d'ovotransferrine a fortement diminué après la mue (11,69 et 5,54 mg/g coquille respectivement). Ces observations suggèrent que les modifications de la matrice organique et de la taille des cristaux pourraient contribuer à l'amélioration de la solidité de la coquille d'oeuf après la mue.

Introduction

The eggshell is a bioceramic material consisting of a mineral part pervaded with an organic matrix (Nys et al., 2001). The columnar crystal forming the palisade layer are anchored on eggshell membranes and are constituted of calcite crystal, the disposition and size of which change across it thickness. It is well established that the amount of material contributes to the mechanical properties of the eggshell but there are also evidence showing that size and orientation of crystal may influence the eggshell mechanical properties (Rodriguez-Navarro *et al.,* 2002). Eggshell texture results, on one hand from competition of crystal growth between adjacent site of nucleation but it is suspected that matrix components influence size and orientation of crystal and therefore contribute to mechanical properties of this biomaterial. Numerous proteins from the eggshell matrix have been recently identified in hens (Nys et al., 2001). Our objective was to analyse any relationship between the amount of components of the eggshell organic matrix and eggshell mechanical properties. That was investigated by comparing eggshell of hens before and after a molt, as it is well established that artificial molt improves eggshell quality (Berry and Brake, 1987; Bar *et al.,* 2001).

Materials and Methods

Fifty nine 78-week-old ISA-Brown laying hens were housed in individual cages, received 14h of light per day and were fed *ad libitum* with a mashed laying diet (formulated as recommended by INRA, Station de Recherches Avicoles). Molt was induced by feeding hens a diet containing a high level of zinc oxide (20g zinc/kg) for 2 weeks and suppressing water for two days. A low calcium cock diet (50g/hen/day) was allocated for 2 weeks before restoring the laying diet. A short photoperiod of 8h light was applied for one month, then was increased until the initial photoperiod. Egg production and egg weights were recorded daily.
Egg size, weight and eggshell quality were measured before and after molting. Eggshell percentage, index, thickness, elasticity, toughness and stiffness were calculated as described by Sauveur, (1988) and Bain, (1990).

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Organic matrix proteins were extracted by demineralisation in acetic acid (20%) of 500 mg of pooled powder shells (4 eggs laid by each hen). These samples were then lyophilised and extracted with an extraction milieu and dialysed as described by Panheleux *et al.,* (2000). Total proteins were then measured by the Bradford method using ovalbumin as a standard. After that, indirect ELISA was used to measure the content of ovotransferrin and ovocleidin-17 in each extract as described by Panheleux *et al.,* (2000).
Statistical analysis: Results were statistically analysed using Stat view software (SAS Institute Inc.). We performed one way analysis of variance for differences before and after molting between eggshell quality parameters and protein content.

Results and discussions

Egg production and egg weights: A positive increase in egg production was observed (0.703 and 0.826 egg/day/hen before and after molting, respectively) in agreement with the observation of an improvement in egg production after induction of an artificial molt by zinc oxide with or without restriction of light (Berry and Brake, 1987; Ramos *et al.,* 1999). Garlich *et al.,* (1984) and Aksoy *et al.,* (1997) also reported an improvement of egg weights and of eggshell quality after the molt. We did not observe any effect of molt on egg weights but our data confirmed the decrease in the percentage of broken eggs (12.5 and 5.6 % before and after molting, respectively).
Eggshell mechanical properties: After molting, we observed an improvement in breaking strength and in eggshell toughness (Table 1). On the other hand, we did not find any effect of molt on eggshell stiffness, elasticity, eggshell index, thickness and eggshell weight. These results therefore indicate that the improvement in eggshell quality or solidity observed after molting is not due to a larger amount of calcium carbonate in eggshell but could be due to ultrastructural modifications.

Table 1: Effect of molt on eggshell biomechanical properties:

	before molt	after molt	P value
Egg weight (g)	65.22	65.83	NS
Eggshell weight (g)	6.24	6.22	NS
Index ($g/100cm^2$)	8.29	8.16	NS
Breaking strength (N)	28.09	33.71	<0.001
Toughness ($N/mm^{3/2}$)	350.17	409.18	<0.0001
Stiffness (N/mm)	142.06	140.29	NS
Elasticity (N/mm^2)	15002.47	15132.78	NS

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Microstructure and crystallographic texture of eggshell are described in the companion paper of Rodriguez-Navarro *et al.,* 2003. Briefly they observed a reduction ($P<0.05$) in the crystal size of eggshell for solid eggs laid after molt. Moreover, the degree of crystal orientation seems to be reduced after molting for more solid eggshells than those produced before molting.

Rodriguez-Navarro *et al.,* (2002) show a negative correlation between the degree of preferential crystal orientation in eggshell of young hens and its breaking strength. That was however not confirmed in older hens, which show more fragile eggshells with very variable structural properties (thickness, morphology of the grains and crystallographic texture). On the mechanical point of view, it is however established, that material with lower size of crystal is more solid and a reduction in degree of orientation is also in favour of better solidity because of the reduction in cleavage plan. We explored therefore the level of organic matrix in these eggshells with different mechanical properties as such changes may explain variations in eggshell fabric.

Organic matrix proteins: Figure1 shows a small increase in organic matrix quantity after molting (18.26 and 20.65 mg/g shell before and after molting, respectively). This limited change may explain the absence of effect on eggshell elasticity but eggshell membranes properties, which are likely to contribute for this parameter, have not been evaluated currently. On the contrary to total protein, we found a sharp decline in ovotransferrin concentrations after molt when compared with the extracted eggshells before molt (11.69 and 5.54 mg/g eggshell before and after molting, respectively). Ovocleidin-17 level was also lower in eggshell matrix extract after the molt (9.83 and 8.87 mg/g eggshell before and after molting). Panheleux *et al*., (2000) did not observe change in total proteins concentration in eggshells of old and young hens. In agreement with our data, they showed a decrease in ovotransferrin concentration in eggshell in young hens when the breaking strength is higher. The same tendency was observed for ovocleidin-17.

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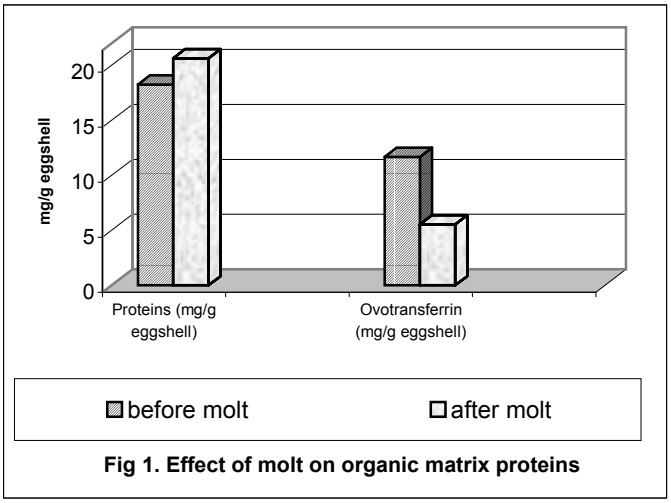

Fig 1. Effect of molt on organic matrix proteins

In conclusion, the differences in eggshell solidity before and after molting are not currently due to an increase in the inorganic part (calcium carbonate) of the eggshell, but to the modification in the ultrastructure of eggshell (size and orientation of eggshell crystals) that coincided with changes in some of the organic matrix constituents. Evidences for a role of the organic matrix in the control of crystallographic and mechanical properties of eggshell are increasing (Nys *et al.,* 2001). Proteins of the organic matrix may influence the organization of the growth of the crystals, their size and their orientation. This hypothesis is reinforced by observations of *in vitro* modification in crystal morphology after introduction of uterine fluid which contain matrix proteins (Dominguez-Vera *et al.,* 2000). However, more information is needed to confirm the relationship between organic matrix contents and eggshell quality and, to know if these proteins can be, eventually, used as markers of eggshell solidity.

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Session 10
Alternative housing systems for egg production

EXPERIENCES WITH PRODUCTION AND EGG QUALITY IN ALTERNATIVE SYSTEMS AND LARGE ENRICHED CAGES FOR LAYING HENS IN THE NETHERLANDS

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Keywords : production, egg quality, aviaries, enriched cages, layers

Abstracts

In the Netherlands aviary systems are used on commercial farms since 1991, enriched cages are only used in experimental units. Aviary hens require more energy for movement, resulting in a higher feed intake, a lower egg weight or both. Egg quality is equal or better than in cages, provided that the percentage of floor eggs is low. The modern aviaries, in combination with good management, showed low percentages of floor eggs. Light programmes appear to be very effective in establishing a low percentage of floor eggs. Production in enriched cages is good and feed conversion ratio can be very low. However, egg quality, and especially cleanness of the eggs need improvement in most types. Not much difference is observed in egg quality between large and small enriched cages, except that the one model with good egg quality was a large enriched cage.

Résumé

Aux Pays Bas les systèmes de volières sont utilisés depuis 1991, tandis que les cages sont utilisés seulement dans les unités expérimentales. Les poules pondeuses dans une volière ont besoin de plus d'énergie pour satisfaire des déplacement plus nombreux, avec comme résultat une augmentation de l'indice de consommation, une réduction du poids de l'œuf où une combinaison des deux. Un système de volière moderne, allié à une bonne maîtrise, n'engendrent qu'un faible pourcentage de ponte en dehors des nids. Des programmes de lumière adaptés sont très efficace pour minimiser le pourcentage de ponte en dehors des nids. Dans les cages aménagées la production est bonne et l'indice de consommation est du même niveau que dans les cages traditionnelles. En revanche, la qualité des œufs, notamment le pourcentage d'œufs sales, doit être amélioré pour la plupart des systèmes. Il n'y a pas de différence

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marquée dans la qualité des œufs suivant cages aménagées, grande ou petite, a l'exception que la seule cage aménagée avec une bonne qualité d' œufs était une grande cage.

Introduction

During the many years that battery cages for laying hens have been used this type of housing has developed into a very efficient system for egg production. Years of development resulted in the optimum combination of cage floor type, floor slope, and egg collection systems. Eggs from caged layers therefore can meet very high quality standards.
The European Directive 1999/74/EC bans cages in the year 2012. From then on only enriched cages or alternative housing systems are allowed. Alternative housing systems are deep litter systems or aviaries, with or without free range possibilities. Both enriched cages and alternatives are equipped with nestboxes to allow hens practising their natural nesting behaviour. This results in a different route eggs will pass before ending onto the egg belt. As the characteristics of an artificial grass mat differ from a cage bottom, and the nest- and cage dimensions differ from the traditional cages, it can be expected that new combinations of floor type and floor slope will have to be realised to guarantee egg quality. On top of that, not all eggs are laid in the nestboxes. Eggs laid onto the wire floors or in the litter are likely to be damaged or soiled. However, with a good lay-out of the system and its different elements a good egg quality should be possible.
In the Netherlands research on aviary systems started in 1981. After several smaller and larger trials this resulted in improvements of the aviary and in 1991 the system was introduced on commercial farms (Van Niekerk & Ehlhardt, 1995). The first three farms were closely monitored on all aspects (technical results, bird health, labour, environmental pollution, economy, etc.). After a period without research on aviaries this was restarted in 2000, following the new EU-directive that came into force. Apart from research in our own facilities many data are collected on commercial farms by both research and extension services.
In 1993 research on enriched cages was started. The first trials focussed on both altering traditional cages and developing new systems. In 2000 a trial with 6 different enriched cages was conducted. Not only small units (8-10 hens), but also large units (20-50 hens) were tested. The most recent trials are done with not beak trimmed hens, because the Dutch legislation will ban beak trimming in cages within a few years. Especially the large units have a higher risk for cannibalism if birds are not beak trimmed. However, they have the advantage of not looking as much as a cage. This may easier lead to acceptance by animal welfare organisations. As no fully equipped

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enriched cage systems are installed on commercial farms yet, research on this type of housing has been conducted only under experimental conditions.
In this paper the experiences with alternative housing systems and large enriched cages are given.

Material and Methods

The first farms with aviaries in the Netherlands were very closely monitored on several aspects. The information was compared with similar information from 50 commercial cage farms (Van Horne, 1996). These farms represented an average of the Dutch farms with laying hens in cages (old, new, large, small), with an average flock size of 29,000 hens (ranging from 7,000 to 69,000). The aviary farms were all managed by experienced farmers, who just had switched over to aviaries. The buildings were mostly new. The average flock size in the aviary henhouses was 16,000 hens, ranging from 4,000 to 26,000 hens. The average number of hens per m2 floor surface of the house was 20 (ranging from 16 to 24) in the aviary houses and 28 (ranging from 13 to 42) in the cage houses. Both cage and aviary flocks were white birds, beak trimmed at 6 weeks of age.
In 2000 a trial with two different aviary systems has been conducted. These systems differed in percentage of wire floor. The Natura-Nova system had 32% wire floor, the Comfort/Compact system had 57% wire floor. Due to differences in available/usable space, number of nests, feeder and perch length, etc. bird density could not be kept exactly equalover the different systems. In the Natura-Nova 13.1 hens per m^2 floor surface were housed, in the Comfort/Compact this was 14.5 hens per m^2. One of the aims of the research was to see how differences in lay-out influence incidence of floor eggs and thus egg quality. The brown birds in this trial were beak trimmed before 10 days of age.
Measures to prevent floor eggs were also investigated in 25 aviary flocks with access to free range (Emous & Fiks - van Niekerk, 2003). Farmers were inquired what measures they had taken to reduce floor eggs. They also gave the average number of floor eggs they had in their flock.
Several enriched cage types have been tested in several trials. These types differ in group size and lay out. All are equipped with perches, nests, dustbathes and at least 750 cm^2 space per hen. Hens in these cages were mostly non beak trimmed, but a few trials were conducted with beak trimmed hens.

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Results and discussion

Aviary hens require more energy for movement. It is therefore not surprising that both in experimental units and on commercial farms feed conversion ratio was higher in aviary hens than in caged layers (Van Horne, 1996; Van Niekerk & Ehlhardt, 1995). In the experimental units the difference was due to higher feed intake in the aviary. Van Horne (1996) found no significant difference in rate of lay, eggs per hen housed and feed intake, but egg weight of aviary eggs was lower compared to cage eggs. The findings of Van Horne (1996) and Van Niekerk & Ehlhardt (1995) are in accordance with each other. The extra energy required for movement in aviaries compared with caged layers is either causing a higher feed intake or, with equal feed intake, smaller eggs or both.

Egg yield is not only depending on production level, but also on egg quality. Both small-scale experiments and commercial flocks showed that in a well equipped aviary henhouse the number of second grade eggs on average is lower than in traditional cages (Van Horne, 1996; Van Niekerk & Ehlhardt, 1995). However, these figures apply only to the eggs laid in the nests. The total amount of second grade eggs will greatly depend on the number of floor eggs, as the majority of these eggs are dirty. Van Horne (1996) observed an average of 4.6 percent floor eggs in commercial flocks and stated, that the total percentage of second grade eggs therefore will not be different for both systems. In a recently conducted inventory held under 25 Dutch aviary flocks with access to free range, average percentage of floor eggs amounted 1.9% (Emous & Fiks - van Niekerk, 2003). Compared to the results of the earlier inventory of Van Horne (1996) one could conclude that lay-out and/or management of aviaries are improved and egg quality in aviaries is now better than in cages. This is confirmed by the fact that in the inventory of Emous & Fiks - van Niekerk (2003) systems that were used for 3 or more production cycles (older systems) had more floor eggs than systems used for only 1 or 2 cycles (new systems; 2.3% versus 1.2%).

The previous results are mainly from hens that were beak trimmed at 6 weeks of age. Beak treatment at an earlier age results in a longer, sharper beak later on and more problems with featherpecking in the laying period can be expected. Feather pecking and cannibalism may influence the number of second grade eggs. In two trials in a deep litter system hens that were beak trimmed before 10 days of age had lower percentages of second grade eggs than hens that were not beak trimmed (10.1 % versus 13.9 %; Fiks- van Niekerk et. al., 2003). The difference was caused by differences in dirty eggs. In the flocks that were not trimmed, more feather pecking and cannibalism occurred and hens used the nests as a hiding place. This resulted in more manure in the nests and thus more dirty eggs. The same effect can be expected with aviary hens as a result of the EU-requirement that allows beak trimming only before 10 days of age. In a trial with two different aviary systems with hens trimmed

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before 10 days of age the average percentage of floor eggs was only 1.3%, but the percentage of second grade eggs was 11.9, which is almost double the highest number found in earlier trials with hens trimmed at 6 weeks of age (Emous et al., 2001; Van Niekerk & Ehlhardt, 1995).
In the quoted inventory held under 25 Dutch aviary-free range flocks measures were listed that were used to reduce the number of floor eggs (Emous & Fiks - van Niekerk, 2003). Table 2 shows that two measures were used in all flocks: frequent collection of floor eggs and electric fencing of places where floor eggs were often laid. Affectability of these measures therefore could not be investigated. Looking at the other measures against floor eggs, it can be seen that some of the very popular measures (e.g. aviary rearing system, distribution of hens over nest rows) appear to be less affective. Light programmes appear to be very effective in establishing a low percentage of floor eggs. It is not possible to distinguish from these figures whether a measure is ineffective or simply not applied properly.
The experiences with enriched cages in the Netherlands are only based on experimental units, as no completely furnished enriched cage is operative on commercial farms. In the experimental units production in enriched cages was good, but if cannibalism occurred this caused a drop in production and more dirty eggs. The most recent research results show that feed intake can be as low as in traditional cages, provided that no feather pecking occurs. Egg quality is often a problem in enriched cages. In table 3 the results of 5 different systems are given. It can be seen that all types have problems with cracked or dirty eggs, except for one model with good results. Apart from this (large) model there is no clear difference between large and small enriched cages. All systems have a good nest acceptance so the problems must be caused in the nests and by the distance eggs have to roll to get onto the egg belt. Nest layout often needs improvement. Also the presence of litter results in more dust on the eggs.

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Table 1: Characteristics of the monitoring on commercial aviary farms with free range

Item	Value	Remarks
Number of farms	17	11 farms with 1 henhouse, 5 farms with 2 houses and 1 farm with 4 houses
Number of flocks	25	from February 2000 until April 2001
Average number of hens	16,400	3 x smaller than 10,000; 14 x between 10,000 and 20,000; 8 x larger than 20,000
Type of hen	4	24 x brown layers, 1 x LSL
Free range flock no. (excl. previous flocks without free range)		7 x 1st - 2nd flock; 11 x 3rd - 5th flock; 7 x 6th - 10th flock

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Table 2: Measures against floor eggs and their effect under commercial circumstances

	Measure applied		Measure not applied	
Management measure	**# flocks**	**% floor eggs**	**# flocks**	**% floor eggs**
Frequent collection floor eggs	25	1.9	0	n.a.
Electric fencing	25	1.9	0	n.a.
Dusk period before start of dark period	14	1.3	11	2.7
Dim lights in morning	14	1.5	11	2.7
More nest surface (>100cm^2/hen)	9	1.3	16	2.4
Extra stairs / perches	4	1.2	21	2.2
Temporarily locking hens up / closing parts litter	5	1.3	20	2.2
Stimulating distribution of hens over length henhouse	13	1.6	12	2.3
Placing hens on tiers for first nights	18	1.8	7	2.4
Rearing in rearing-aviaries	21	2	4	2.1
Water in front of nestboxes	17	2	8	2
Stimulating distribution hens over different nest levels	19	2.1	6	1.8
Thin layer of litter	23	2.1	2	1.1
Sloping wire floors	21	2.2	4	1.1

Table 3: Percentage of eggs laid in the nests and percentage of second grade eggs for different types of enriched cages (non beak trimmed LSL).

Cage model		**% eggs**	**% second grade eggs**		
Hens/cage	**Model no.**	**in nest**	**Total %**	**% dirty**	**% cracked**
< 20	Model 1	95.2	16.2	13.1	2.0
	Model 2	> 97	21.2	18.2	1.4
≥ 20	Model 3	99.2	22.2	16.1	4.3
	Model 4	98.2	16.6	13.1	1.3
	Model 5	98.6	6.6	4.7	0.3

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EXPERIENCES OF PRODUCTION AND WELFARE IN SMALL GROUP CAGES IN SWEDEN

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Keywords : Furnished cages, health, welfare, production, egg quality

Abstract.

Furnished cages have quite a long experience in Sweden with research since the 1980's and finally, commercial introduction in 1998. The use of smaller groups until now (≤10/cage) is mainly because beak trimming is prohibited thus, reducing pecking problems more likely to occur in larger groups. After compulsory and individual testing in practice for animal welfare evaluation four different models are now approved. Today about 1,5 million hens (30%) of the national flock are kept in these systems. The vast majority of investments represent various models of the EMC-concept, i.e. with the nest and litter box placed at on side of the cage and perches parallel to the feed trough. From the comprehensive data accumulated in recent research and in the commercial national tests, it can be concluded that normal production and mortality are obtained. Considerable variation within models still occur, e.g. in egg quality due to details of design.

Résumé.

Les cages aménagées sont expérimentées en Suède dans le cadre d' un programme de recherche depuis 1980 et ont finalement été introduites sur le marché en 1998. L'usage de petits groupes (≤10/cage) est recommandé principalement à cause du problème de picage, exacerbé par l'interdiction du débecquage. En effet, la fréquence du picage augmente beaucoup dans les groupes de grande taille. Des tests obligatoires et individuels en condition pratique destinés à évaluer les bien-être des animaux ont permis l'approbation de quatre modèles différents. Aujourd'hui, environ 1,5 millions des poules (30%) sont gardées dans ces systèmes de cages aménagées. La grande majorité des investissements représentent des modèles différents du concept EMC, par

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exemple, des systèmes avec le nid et le bac à poussière placés sur le côté de la cage et les perchoirs parallèles à la mangeoire. Les résultats récents issus de la recherche et des tests nationaux en conditions commerciales, démontrent que la production et la mortalité obtenues sont normales. Cependant, des variations considérables en fonction des modèles de cage, notamment pour la qualité des œufs sont observés.

Introduction

In 1988 the Swedish animal Welfare Ordinance (SFS, 1988) was altered saying that in 10 years time all cages for laying hens should be banned. However, the Standing Committee for Agriculture and Environment mentioned four different conditions to be fulfilled by the alternative systems; Animal health and working conditions for operators must not be impaired, beak trimming (prohibited) must not be introduced and medication must not increase. Also, the compulsory Swedish testing of the new systems in commercial production must be carried out. A fifth criteria was in fact, added in 1995 when Sweden joined the EU; Swedish egg production should not be out-competed by foreign cage egg production.
After this decision a period of uncertainty among scientists, producers and advisors followed. Comprehensive research that had already started with new systems and others to follow also included the Swedish compulsory testing of e.g. aviary systems. In brief, the procedure of testing New Technique evaluates animal welfare traits and production mainly in the field by comparing traits with preset limits of health and production parameters.
Still in 1997 about 80% of the laying flock was in conventional cages and the tests as well as research had failed in finding alternative systems that passed the four criteria given by the Committee eight years earlier. The main objections were feather pecking and cannibalism, air conditions, parasitic disease and special health aspects like bumble foot. Although, progress was made in several of the mentioned traits there was an 80% parliamentary majority for modifying again the Animal Welfare Ordinance of 1988. Thus, in 1997 Parliament voted for another new wording in the Ordinance (SFS, 1997), i.e. that only such cages could be used having a litter box, perch and a nest allowing birds to perform natural behaviours.

Furnished cages enter the commercial scene

In the late 1980's and during the 1990's there had been comprehensive studies at Funbo-Lövsta Research Centre at SLU in Uppsala on various models of furnished

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cages starting with the so called Get-Away cage (e.g. Elson 1976) turning later into research on smaller groups of the EMC-concept, i.e. with the litter bath upon the nest at the far side of the system and the perch parallel to the feed trough (Abrahamsson, et al., 1995 and 1996; Abrahamsson and Tauson, 1997). This work also involved studies on details of designs that could be used in floor hen housing, like designs of nest material or perches. However, during the late 1990's along with that results from practice on aviary systems were not that successful in several respects, studies in this system more and more got into research with a possible aim to have it in commercial production eventually. In the autumn of 1998 the first farms with the so called « Comfort cage » by the Victorsson AB company was introduced after six years of research and development at Funbo-Lövsta starting with the 5-bird EMC-concept in small scale studies used by mainly Mike Appleby and Barry Hughes (Appleby & Hughes, 1995). The main modifications to this system concerned the increased group size to 8 birds/cage for economic reasons, the nest design and material, the litter bath and its closing mechanism, perch design and of course the total mechanical development of it, i.e. with automatic distribution of feed, manure removal and egg collection system. This model (Fig. 1) got its approval for commercial sale in 2000 (Tauson and Holm, 2002a).

In 1997, a new concept - the Big Dutchman Aviplus - with the nest and litter belt facilities in the rear and made for 10 birds (Fig. 2) started to develop. In 2001 it got its Swedish approval. Today there are four different companies selling furnished cages in Sweden (Big Dutchman, Hellmann Poultry, Triotec and Victorsson). The total number of layers is about 1.5 million birds, which representing almost 30% of the total flock. Thus, today Sweden is the first country where furnished cages are spread commercially. More than 85% of birds are SCWL:s. While the exchange of the conventional cages is still under process after individual quality based exemptions were given until maximum 2004, the number of farms with new equipment is still increasing. The other alternative systems installed are the traditional deep litter systems and multi-tiered aviaries. The majority of the furnished cages models are different EMC-concepts and less of the Aviplus.

Still if R & D in furnished cages have been going on for some time it is still a new system for many manufacturers and egg producers. Hence, ongoing work on details and new concepts of design is important (see example in Fig. 3). In the present paper I will try to give some examples of how R & D have progressed.

Production, mortality and fcr in small group cages

At quite an early stage of research it was shown that it was possible to obtain good production levels in the furnished cages (Appleby et al., 1993). Table 1 gives recent

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data on some production traits at a comparison of three different cage models at Funbo-Lövsta Research Centre while Figs. 4-5 give mortality data from the commercial flocks of Victorsson and Aviplus. Data are compared with the Danish commercial production figures registered from conventional 600cm^2/cage floor space per hen during 2000-2001 (The Danish Poultry Council, 2002).
Mortality in some flocks has been negatively influenced by outbreaks of Leucosis in one main hybrid in Sweden and by Coccidiosis at early ages. The latter occurred only in the Aviplus because of manure build up at non-optimal perch locations. Apart from that, it can be seen that mortality rates (Fig. 4-5) in the commercial flocks have been quite acceptable although, sometimes there is a tendency for a slight increase in the latest periods of production possibly due to the larger group size compared to the conventional cage.

Table 1. Production data (20-80 w.) from the latest study at Funbo-Lövsta with two genotypes and two kinds of furnished cages and a conventional 4-hen cage. Leucosis appeared in the LSL birds. (Tauson and Wall, 2003).

Model	**"Aviplus" (Big Dutchman)**		**"Comfort cage" (Victorsson)**		**Conventional (Triotec)**	
Hybrid	**LSL**	**LB**	**LSL**	**LB**	**LSL**	**LB**
Parameter						
Birds/cage	10	10	8	8	4	4
Laying, %	89,1	87,6	88,3	86,7	88,8	87,1
Egg weight, g	63,6	66,0	63,9	66,3	63,9	66,9
Eggmass, g/hen day	56,7	57,8	56,4	57,4	56,7	58,2
Eggmass, kg/hen housed	22,8	23,9	23,1	23,6	23,0	23,9
Mortality, %	8,7	4,3	4,2	5,0	5,9	4,9
FCR, kg feed/kg egg	1,92		1,92		1,94	
Feed cons., g/hen day	110		109		112	
Cracks, %	1,8	3,6	2,2	3,6	1,8	2,7
Dirty, %	17,5	7,9	5,9	4,0	9,2	4,9

From the station results (Table 1) and from the commercial farms it can be concluded that production, mortality and feed conversion are now a days very similar to conventional cages. Regarding feed consumption there has been higher FCR:s in furnished cages than in conventional cages reported earlier. In fact, there are factors in this system that would be expected to increase as well as decrease energy requirements. The larger area of the cage, activities like stepping up onto perches or dust bathing would be of the former kind while resting close together at the perch would save heat losses and reduce energy requirements for locomotion. The fact that plumage condition is essential in saving bird body heat losses in many European countries (Peguri and Coon, 1993) should also be considered. On average the plumage condition reported from furnished small group cages in Sweden has been better than in

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conventional cages (see below). However, feather pecking especially, in non-beak trimmed medium heavy genotypes is more apparent than in SCWL: s. In a long term study in a commercial farm average production in the Comfort cage has been 3,5% and FCR 4,4% higher than in deep litter birds (Tauson and Holm, 2002b).

Egg quality

An obvious improvement in furnished cage design during the recent years has been in external egg quality (dirties and cracks). Still there is quite a difference among models mainly due to lack of experience in the manufacturing industry but also, need for more research activities in this trait. Nest design and location, bird behaviour in it, perch location and egg cradle construction are several examples where egg quality may be significantly affected. Behaviours such as staying over night in nests may dirty the nest pads, which can increase the proportion of dirty eggs.

In conventional cages the eggs are rolling out of the nest spread over the whole width of the cradle while in furnished cages the eggs are laid in the nest, which – at least in the EMC-concept – implies higher concentration of egg location after roll-out. Hence, the risk for collision between eggs is far greater in the cradle. Thus, this resembles the effect shown on cracked egg proportion in deep vs. shallow conventional cages (Hughes and Black, 1976). Also, if eggs are laid far up in the nest the speed of the egg at rollout is greater. Hence, if birds lay with their cloacae towards the nest opening or the other way around is an important observation. In the Aviplus the rolling out of the egg is not so critical because the nest is wide and shallow (Table 1). However, the inspection of the egg collection belt requires extra attention due to the location in the dark at the far rear of the system. The matter of birds laying from the perch has not been reported as being a problem in Sweden.

The use of either prolonged nest curtain plastic strips at the end of the nest or an « egg saver » wire installed to be raised/lowered every 10-20 min during peak of daily lay, both have dramatically reduced cracked egg proportion (Wall and Tauson, 2002). As can be seen in Table 1 the proportion of cracked eggs is at a low level today where considerations like the mentioned ones have been taken.

Regarding the proportions of dirty eggs at ocular inspection normally, if hygienic conditions are good depending mostly on nest design and perch location, the recent data suggests that an acceptable and low proportion can be achieved (Table 1). However, the Aviplus model has most often been shown to give more dirty eggs than the EMC-concept. Possibly, having the extra facilities at the rear makes light intensity and thus locomotion of birds less implying trampling through the cage of manure less effective. A supportive observation from practice is also that the width of aisles between cage rows may affect eggshell hygiene in this system.

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The economic result on final egg price to the producer after delivery to the packing station depends largely on the proportion of cracked and dirty eggs obtained from a specific housing system, e.g. from results reported above. The hygienic conditions as regards microbiological load of the egg as well as on its shell may also be important in a wider sense. In an ongoing EU-project - "Eggdefence" - this has been studied. From preliminary data at 27 weeks of age of birds it was shown that the bacterial load on eggshells was low and acceptable on eggs from both conventional cages and furnished cages (Victorsson – see Fig. 1) (Tauson et al., 2003). However, although the proportion of eggs with Enterococcus was not different between the two systems, the average amounts of bacterial load on eggshells were higher in the latter system. Further studies within this scientific cooperation as well as others will hopefully look for info on the hygienic quality of eggs also from other alternative systems e.g. aviary systems and ecological production in relation to furnished and conventional cages.

Use of facilities and management

The use of nests in furnished small group cages is normally between 90-100% (Tauson and Holm, 2002) if a well designed and located nest is presented. In practice the amount of eggs laid outside the nest is most often at only 1-3%. This well illustrates the bird's appreciation of this facility - possibly the main behaviour most lacking in a conventional cage. The use of perch(es) depends on the arrangement of it. The 1999 EU welfare minimum requirement states 15 cm perch length per bird. In Sweden before, it was 12 cm. Increasing it to 15 cm most often implies that a cross of perches has to be made somewhere. In fact, this means that birds cannot use that specific area for perching (Tauson et al., 2003). The average perching seen in the commercial testing in Sweden varies between 70-95% at night (Tauson and Holm, 2002a) implying perching also representing a behaviour intensively performed.
The litter box by Swedish directive (SJVFS, 2003) opens at least eight hours after lights on and is left open for at least 5 hours. This implies that there is normally no problem with eggs laid in the litter area. The use of the litter box depends on many factors where location and thus, easy access as well as size are important factors. Thus, due to its lower position the Aviplus litter trough is easier to climb into than the higher located litter box in the EMC-concept. Also genotype has an effect. Thus, brown medium heavy hybrids normally use the litter more than SCWL: s. Recent data also suggest that birds reared with litter use this facility more than birds reared in cages (Wall and Tauson, 2003).
Thus, one can say that the birds very intensively use the two facilities nest and perch but that the use of the litter is more variable not only due to the factors mentioned above but also with great variation among individuals. This raises another important

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issue; the fact that all birds in a flock or in a cage do not necessarily have the same need for litter? For some birds however, it might be critical at sexual maturity due to build up of stress. Hence, if litter was not to be present as a pecking, foraging or dust bathing or only as an occupational device, the most sensitive birds may cause severe problems e.g. as peckers or other stress releasing symptoms directed towards the remaining group of birds.
The furnished cage is a new system for commercial use. After the final tests in Sweden new ones are entering the market. One example is the location of the nest, still with egg out-rolling at the feed trough, e.g. the EU625-model (Fig. 5). In this concept also used by other manufacturers the egg out-rolling is located over an open area where birds move around and eventually also feed.
Thus, special attention needs to be taken to the management of functions in different models of furnished cages. The litter opening/closing just mentioned is one. Litter may be filled either by hand in smaller operations (<8-10.000 birds) or by trucks run in the aisles with tube injectors located in front of each litter box. An auger tube in the rear partition between two opposite cages with litter let down through vertical short tubes into the litter box is also used. The litter belt in the Aviplus model is another solution. The most common litter used is sawdust. Wood shavings may also be seen but is less appreciated by the birds due to its coarser structure not entering the plumage as well.
The regular manure removal normally carried out by belts needs two runs per week in order not to have piles of manure build up on the belt under the perch arrangement. Even if the build up may not reach the floor to cause dirty eggs it can be reached by the birds at pecking through the floor possibly leading to coccidiosis or by toes/claws of birds. The latter may decrease egg quality. The importance of giving a feed composition providing a manure with acceptable dry matter content should be emphasised due to the fact that a wet manure would be even more sensitive to the hygiene of a furnished cage than a conventional one.
Since the furnished cages are higher than conventional ones they are more difficult to inspect. Hence, in some models it is motivated to use a travelling cart run at rails on the lowest tiers which the operator stands on and by this can inspect all tiers of three (max. allowed e.g. in Scandinavia). The inspection is especially important in models where the facilities like nest/litter are placed at the rear, e.g. in the Aviplus.

Welfare and health

In the evaluation program birds are scored for health characters at 35 and 55-65 weeks of age (Tauson and Holm, 2002a). Among the health traits are plumage and foot condition, pecking wounds on comb and around the cloacae/back region. At these occasions birds are also weighed and certain behaviours are registered, especially

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those linked to aggressiveness and/or feather pecking. The plumage condition scores give the degree of feather pecking and are graded as % of birds with naked areas. Especially, in countries where beak trimming is not allowed, e.g. Sweden, Norway and Finland, this is normally may be a serious problem especially in brown medium heavy birds and in larger flocks on the floor. The problem in the small group cages with SCWL birds has been moderate with a low proportion of birds with naked areas (>5 cm in diameter) although, there are flocks with higher numbers. These data are still in general considerably better than in most of the aviary systems evaluated with the same system during the mid 1990´s (e.g. (Ekstrand, et al., 1996). In a field study two flocks in a single tier deep litter system were compared with parallel flocks of the same hatch and genotype in the first models of commercial furnished Victorsson cages (Tauson and Holm, 2000b). The results in the furnished cages were found very competitive in several respects to this quite common alternative system. For instance plumage and foot condition (bumble foot syndrome), pecks at comb and at rear were less common in the furnished small group cages than in the deep litter system.

The number of pecks registered in different categories in the small group furnished cages has normally been low but occasionally with certain genotypes more frequent. As the litter distribution system as well as the opening door mechanism have been subject to considerable malfunction in some models, litter has not been present as intended in these cases. In some farms and with some genotypes pecking has been more frequent than in others. Another significant effect on bird's health registered is the increase in bone strength (Abrahamsson and Tauson, 1997) compared to conventional cages probably due to the increased locomotive activity in the larger area and to the use of the extra facilities described earlier.

Due mainly to the ban on beak trimming the group size in furnished cages will most likely remain small/moderate although, a larger group means a larger cage with even more space to move around in and more possibilities to introduce nests and perches. However, the use of a partition at the rear with pop-holes in it in group sizes up to 16 birds per cage has been shown to work well with good use of the possibility for birds to move away and change apartment (Wall et al., 2002)

Conclusions

According to research as well as commercial tests in Sweden since 1998 there are small group furnished cages that are serving as viable alternatives to the conventional cages. Mostly white genotypes have been used. Production traits as well as welfare measured as health are competitive and often better than in other alternative systems studied parallel. The use of facilities like the nest and perch is very intensive while the use of the litter is more variable. Because R & D is spread differently between

manufacturers and that the system in general is still a new one, there are still differences in results between concepts and manufacturers. It is concluded that further improvements in nest design, perch location and litter handling may still be potentials for increasing the competitiveness of this system. Due mainly to the ban on beak trimming in Sweden the group size in furnished cages will most likely remain small to moderate.

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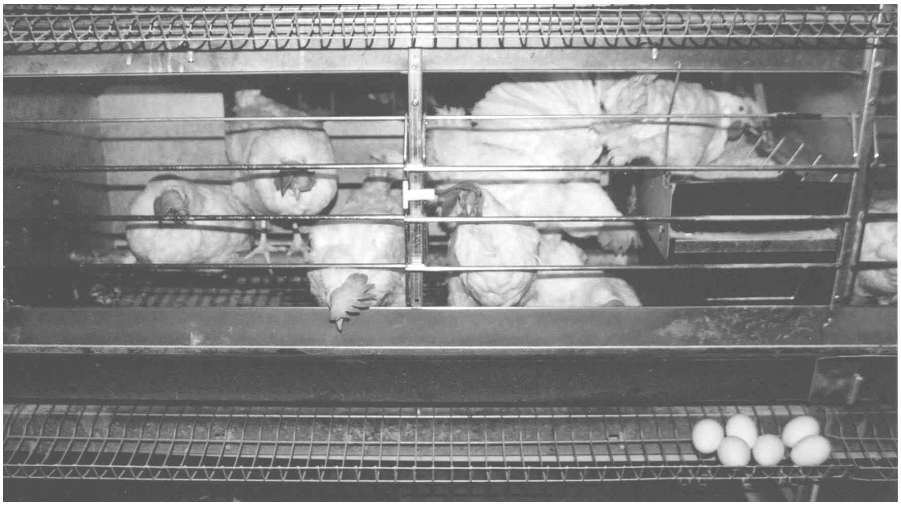

Fig. 1. “Comfort cage” (Victorsson) for 8 birds illustrating use of nest, litter box, perch and wing stretching in 45 week LSL birds.

Fig. 2. Aviplus Big Dutchman furnished cage for 10 birds with auomatic litter belt and bath and nest at the rear. Perches paralell to the feed trough. Egg out-rolling inwards the cage.

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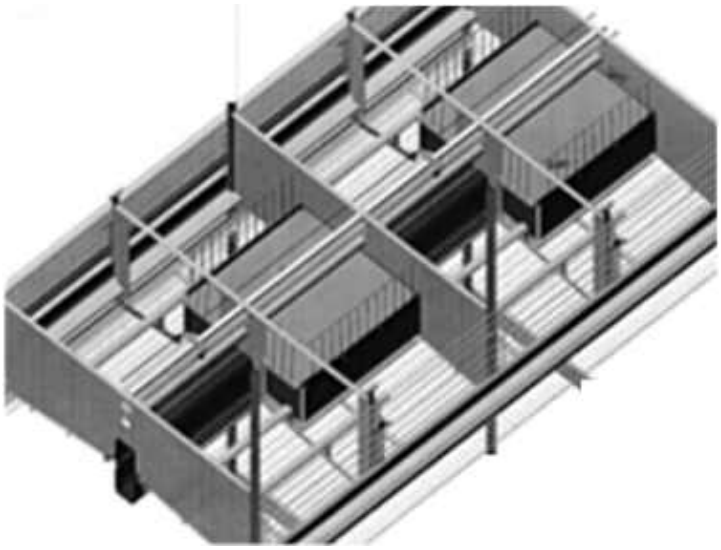

Fig. 3. Big Dutchman furnished cage EU625 (4 cages) for 10 birds in each with nest and litter box placed at the rear and parallel to the partition. (used but not tested in Sweden).

Fig. 4. Accumulated mortality in evaluation program of commercial flocks in 12 farms in Sweden with Victorsson furnished 8- hen cages in relation to accepted limit (9%) and Danish commercial field data on conventional cages 2000/01.

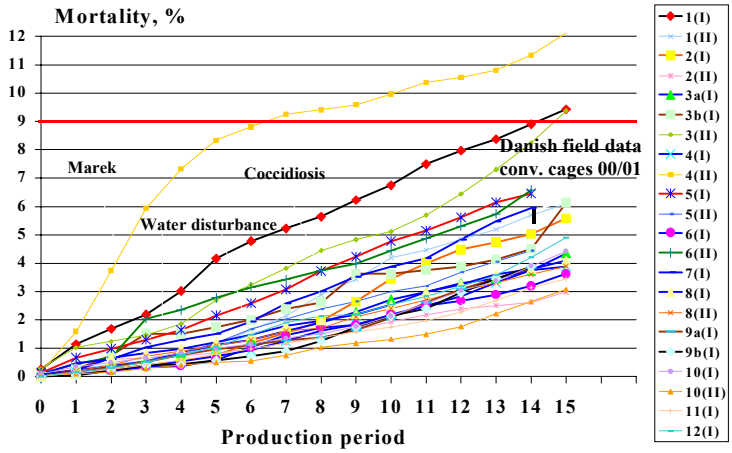

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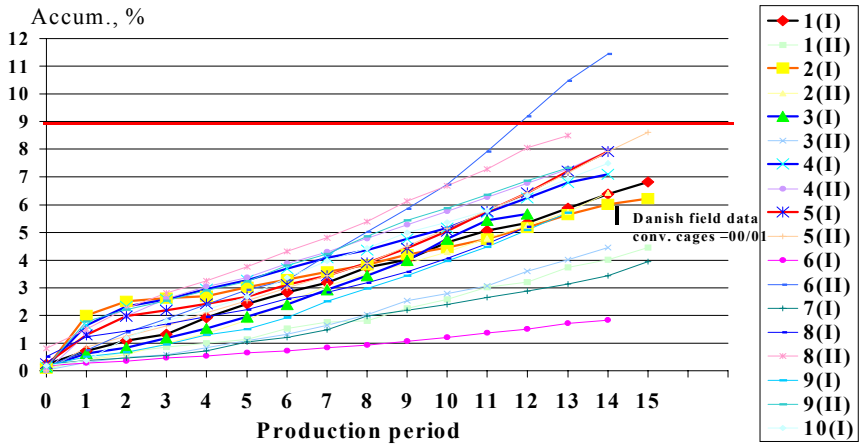
Fig 5. Mortality in testing program at 10 commercial farms in Sweden with Big Dutchman Aviplus, August 1999-January 2003 in relation to accepted max. level (9%) and Danish conventional cages in practice.



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INFLUENCE OF ALTERNATIVE HOUSING SYSTEMS ON TABLE EGG QUALITY

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Keywords : alternative housing, cages, egg quality, farming method

Abstract

The paper presents an updated review about the influence of alternative housing systems on table egg quality. The subject is particularly relevant in view of the incoming new European regulation banning conventional cages for layer poultry. Further in-depth and statistically robust investigations are required to give sound evidence of the advantages/disadvantages of alternative housing systems on overall egg quality.

Résumé

Le travail présente l'état des connaissances sur l'influence des méthodes alternatives d'élevage des poules pondeuses sur la qualité des œufs. Ce sujet est d'actualité en vue de l'abolition des cages traditionnelles prévue par la nouvelle réglementation Européenne. Pour avoir des donnés sures concernant l'influence des méthodes alternatives sur la qualité des œufs des études approfondis, avec traitement statistique des résultats, sont nécessaires.

Introduction

Alternative housing systems are, in our presentation, all housing systems, including organic production, other than cages.
The alternative eggs market grows as an answer to the needs of the consumer, concerned by food quality and health and with an increasing interest for animal welfare and environment protection. Alternative eggs increase the consumer choice options and

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renew the interest for the product egg in a market where, although pro-capite world consumption (about 142 eggs/person/year) increased 20% during the last 12 years, egg consumption is increasing in only 30 countries (including Italy; Gillin, 2002). In USA and France, in 1995, alternative eggs represented 3-5% of table eggs (Patterson *et al.*, 2001).
To allow consumer's identification of farming methods used for class A eggs production, European legislation mandates their indication on packs or eggs shells starting from 2004 (EC, 2001a). The codes and terms specifying farming methods are 0 for organic production, 1 for free-range, 2 for barn and 3 for cages (EC, 2001b, 2002). Minimum requirements of poultry establishments for the various farming methods must comply with Directive 1999/74/EC.
This paper presents an updated review about the influence of alternative housing systems on table egg quality.

Discussion

The first comprehensive review comparing eggs from caged hens and from alternative housing methods has been completed by Sauveur (1991). The Author stated that, apart from the problem of comparing results from different studies, some researches are not rigorous, because they are not performed with hens of the same breed, born the same day in the same flock and with identical feeding; furthermore, free-ranging hens have access to non-controlled "snacks" such as grass, worms, insects, etc. Caution in the interpretation of the results is therefore a must.
Egg weight. Sauveur (1991) reported contrasting results among studies concerning egg weight; recent works, instead, confirm that this character is increased by cage housing (Mohan *et al*., 1991; Pavlovski *et al*., 1994a, 1994b, 2001; Muthusamy e Viswanathan, 1999).
Eggshell hygienic quality. Sauveur (1991) concluded that there is a higher eggshell contamination trend in eggs from non-caged hens, but that good management in alternative farming, such as frequent egg collection from nests and disposal of ground-laid eggs may control this problem.
A smaller incidence of dirty eggs in cage housing with respect to alternative housing was reported also by Pavlovski *et al.* (1994b) and by Abrahamsson and Tauson (1995). Hauser and Fölsch (2002) tested air quality and hygienic status of eggshells in four different farming systems during three seasons, but did not find significant differences, notwithstanding different dust and ammonia concentrations in the environments. Shell contamination was lowest in cage and highest in free-range systems: $2x10^6$ vs $2.7x10^7$

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ufc of total microorganisms, $1.7x10^2$ vs $3.3x10^3$ ufc of molds/yeasts, $1.7x10^3$ vs $5.2x10^4$ ufc of coliforms.

Shell thickness. The results are variable: e.g. Mohan *et al.* (1991) observed a thicker shell in cage eggs compared to deep-litter housing. Later works, instead, did not find significant differences among cage, deep-litter and free-range systems (Pavlovski *et al*., 1994a) or between cage and deep-litter (Muthusamy e Viswanathan, 1999). Pavlovski *et al.* (2001), analysing eggs from three different systems including cage, detected thicker shells in aviary system and thinner shells in free-range farming. On the other hand, Leyendecker *et al.* (2001b) reported thicker shells in free-range eggs, in a study including also cage and aviary systems. A higher percentage of broken and cracked eggs was also observed in cage housing (Leyendecker *et al.*, 2001a).

Albumen height and Haugh Unit. The reports are contrasting. Pavlovski *et al.* (1994a) described better albumen quality in eggs from cages (79,8 HU), with the poorest results coming from deep-litter (75.96 HU). Muthusamy and Viswanathan (1999), studying eggs from five cage and five deep-litter farmings, detected higher HU in the eggs from cage hens. Other Authors, instead, did not observe differences between cage and free-range eggs (Gittins e Overfield, 1991) or between cage and aviary eggs (Abrahamsson e Tauson, 1995). Two recent works comparing eggs from cage, aviary and free-range systems had opposite results: in the first, better albumen quality was evident in aviary system (Leyendecker *et al.* 2001b), while in the second albumen quality was superior in free-range eggs (Pavlovski, 2001).

Albumen height is affected by egg freshness: thus, time from deposition to analysis and hen permanence time on the nest are relevant sources of variation, along with age of hens, in different studies. Interestingly, other egg freshness indices, independent from hen age and thus with smaller natural variation, exist. Indeed, furosine analysis (Hidalgo *et al.*, 1995) and test of the albumen-tetramethylbenzidine reaction (Rossi *et al.*, 2001) could also be used to decrease, in future studies, the sources of variation impairing the evaluation of housing system effects on internal egg quality.

Meat and blood spots incidence. Results are contrasting. Leyendecker *et al.* (2001b), studying eggs from Lohman Tradition hens, and Pavlovski *et al.* (1994a) observed fewer meat and blood spots in free-range eggs. On the other hand, no differences were observed in eggs from Lohman Selected Leghorn under different housing systems (Leyendecker *et al.*, 2001b), or in eggs from cage and aviary (Abrahamsson e Tauson, 1995)

Vitamins, fatty acids and cholesterol. Gittins and Overfield (1991) detected higher B12 contents in free-range (2.21 µg/100g) versus cage (1.56 µg/100g) eggs; no significant differences were found for other vitamins or fatty acids. About fatty acids and cholesterol composition, Sauveur (1991) reported only a tendency, rarely significant, in the increase of linoleic acid and cholesterol in alternative systems eggs.

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Organoleptic traits. Sauveur (1991) concluded that no significant differences exist among cage eggs and industrial alternative eggs. In small farm free-range eggs the results are more variable, as a consequence of less controlled feed.

Others. Lysozyme activity, evaluated by Swierczewska *et al.* (2002) in eggs from free-ranging hens, fed with farm feed as well as in eggs from "intensive" systems (litter and cage, fed with industrial mix), was significantly higher in free range animals. Cage system eggs showed the lowest ability of ovomucoid and ovoinhibitor to inhibit tripsine and chimotripsine (ovoinhibitor only).

Egg quality in modified cages

Little is known about egg quality of hens housed in modified cages. Development and utilisation of modified cages is different in different countries: in Sweden, where conventional cages are forbidden since 1999, the experience in the commercial utilisation and in the assessment of the different types of modified cages is higher (Tauson, 2001). An increase in cracked and dirty eggs are the major changes as a consequence of the presence of nest, litter and perch in furnished cages. However, with the addition of some devices, the proportion of cracks may be decreased (Wall e Tauson, 2001) and good management practices may minimise dirty eggs incidence (Tauson, 2001). However, the development of this kind of cages is just beginning and production and quality results are continuously updated (Tauson, 2001).

Alternative egg quality on the market

Evaluation of the quality traits of alternative eggs is a deserved answer to the expectations of the consumers, willing to pay an extra price for eggs perceived as safer, fresher, healthier and tastier. In this perspective, distribution and retailing practices, not directly related to the housing system, could play a major role on egg quality.

Fatty acids composition of conventional eggs and of five commercial brands of specialty eggs in USA was compared by Cherian *et al.* (2002): no clear influence of housing system on lipid composition could be argued from their results.

A US study on the quality of supermarket purchased eggs (390 conventional egg samples and 246 specialty egg samples; Patterson *et al.*, 2000), shows that specialty eggs satisfy in a lesser degree the classical quality standards (CEE, 1991; USDA, 1995). Specialty eggs (Table 1) were on average older, based on carton pack date, and had a lower albumen height, with lower HU values and a higher proportion of <55 HU eggs. While the proportion of cracks was similar, the proportion of broken eggs was three times higher in specialty vs traditional eggs.

Within the specialty eggs group (Table 2), designer eggs (both "all vegetable diet" and "nutritionally altered" eggs) had internal quality (albumen height 4.8 cm, and average HU 65.5) similar to conventional eggs, but were the oldest eggs, as evidenced by packaging date, along with organic eggs. Organic eggs had the poorest internal quality, while fertile and welfare-managed hens eggs were less old but had HU values of 62.2

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and 61.2, respectively. No differences were detected for cracks, but the proportion of broken eggs was highest in organic and welfare-managed hens eggs.

Conclusion

As a conclusion, a strong influence of housing system on table egg quality is missing. Further in-depth and statistically robust investigations are required to give sound evidence of the advantages/disadvantages of alternative housing systems on overall egg quality. Dirty and cracked eggs proportion may be reduced with good management and changes in planning, positioning and/or materials of the housing devices. The significant genotype-housing system interaction reported by Leyendecker *et al.* (2001 a,b) for some egg traits may suggest that sometimes the conclusions about housing influence on egg quality should also consider hen breeds. The absence of literature references about the influence of the organic production system on egg quality suggests the need to investigate in this direction. Finally, distribution and retailing practices also play a major role in affecting the quality of marketed alternative eggs. An increase in the alternative eggs market could contribute to improve product quality as a consequence of higher turnover and competitiveness.

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Table 1. Characteristics of specialty and conventional eggs market in USA (Patterson et al., 2001).

Item	Specialty egg	Conventional egg	P-value
Age (d)	16.5	11.7	0.0001
albumen height (mm)	4.7	5.0	0.0001
Haugh Unit (HU)	63.8	67.5	0.0001
HU < 55 (%)	16.3	10.6	0.0001
cracks (%)	5.4	5.7	0.3437
leakers (%)	1.0	0.3	0.0191
price mean ($/dozen)	2.18	1.23	0.0001
price range ($/dozen)	0.88 – 4.38	0.39 – 2.35	

Table 2. Characteristics of specialty eggs marketed in USA (Patterson et al., 2001).

Item	All vegetable diet	Welfare-managed	Nutritionally altered	Fertile	Organic
% of eggs	45.4	22.1	19.9	6.5	6.1
age (d)	17.6^{a}	11.0^{b}	19.0^{a}	12.5^{b}	18.5^{a}
albumen height (mm)	4.8^{a}	4.6^{ab}	4.8^{a}	4.4^{b}	4.1^{c}
Haugh Unit (HU)	65.9^{a}	62.2^{ab}	65.2^{a}	61.2^{b}	57.6^{c}
HU < 55 (%)	9.4^{c}	22.4^{b}	13.1^{bc}	19.0^{b}	38.6^{a}
cracks (%)	4.3^{ab}	7.0^{a}	5.0^{ab}	2.2^{b}	8.6^{a}
leakers (%)	0.6	1.0	0.4	2.2	3.6
price mean ($/dozen)	2.19^{b}	2.29^{b}	1.81^{c}	2.24^{b}	2.72^{a}
price range ($/dozen)	1.79 – 3.19	0.88-2.89	1.42 – 2.69	1.89 – 2.39	1.89– 4.38

[a-c] Means within a row with no common superscripts differ significantly (P<0.05)

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COMPARISON OF EGGSHELL QUALITY AND HYGIENE IN TWO HOUSING SYSTEMS / STANDARD AND FURNISHED CAGES.

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Keywords : furnished cages, eggshell, quality, hygiene, microflora.

Abstract

The consequences of the furnished cages on the hygienic properties and eggshell quality of the eggs produced in comparison to conventional cages have been investigated. At 28 and 47 weeks of age, the proportion of dirty, cracked and broken eggs was generally significantly higher in furnished cages as well as bacterial eggshell contamination (total mesophilic aerobic bacteria) at 27 and 60 weeks. Mechanical characteristics of eggshell are not significantly different except for eggshell thickness, higher in furnished cages at 47w. Nevertheless, important differences are observed from a manufacturer to another.

Résumé

Les conséquences des cages aménagées sur les propriétés hygiéniques et mécaniques de la coquille des oeufs en comparaison avec des cages conventionnelles ont été examinées. A l'age de 28 et 48 semaines, la proportion d'œufs sales, fêlés et cassés était généralement significativement plus élevée dans les cages aménagées ainsi que la contamination bactérienne de la coquille (bactéries totales aérobies mésophiles) à 27 et 60 semaines. Les caractéristiques mécaniques de la coquille ne sont pas statistiquement différentes sauf pour l'épaisseur, plus forte dans les cages aménagées à 47 semaines. Cependant des differences importantes peuvent exister d'un fabriquant à l'autre.

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Introduction

Just after laying, the content of the egg from a healthy hen is generally sterile nevertheless, eggshell surface will be rapidly contaminated by environmental bacteria present in faeces, dust or breeding material (Protais *et al.* 1989). Some of these bacteria may be pathogen for humans and able to contaminate egg content. In the 1999 EU directive, it has been decided, to improve animal welfare, that if cages have to be used after 2012 for laying hens, they should be of the furnished design. The objective of the present work was therefore to analyse the consequences of the furnished cages on the hygienic properties and eggshell quality of the eggs produced in furnished or conventional cages from two manufacturers.

Material and Methods

Experimental design : 1992 laying hens (Isa brown strain) were housed in 4 different cage models between 18 and 70 weeks of age with 15 h of light from 2h00 AM to 5h00 PM and fed *ad-libitum* with a standard feed (CM 2800 Kcal, CP 16.3%, Ca 3,6%, available P 0.3%). The cage units were of two categories : conventional cages (*C.*.) with only access to feeders and drinking pipettes and furnished cages (*F.*.) with perch, nest and litter box. They were supplied by 2 different manufacturers (A and B). The two standard cage models provided a minimum surface of 550 cm2 per bird (EU 2005 directive 1999/74/CE) and differed by the number of hens per cage : 96 cages with 5 hens/cage (*C.5A*) or 108 cages with 6 hens/cage (*C.6B*). The two furnished cage models provided a minimum surface of 750 cm2 per bird (EU 2012 directive 1999/74/CE) and differed mainly by the disposition of the furniture and the actual effective surface available for the hens : for *F.15A*, 25 cages with 15 hens/cage (1,5 m^2) and the litter (Astroturf carpet MonsantoTM) close to the nest, for *F.15B*, 36 cages of 15 hens (1 m^2) with the litter at the opposite of the nest. The two conventional cage units were housed in the same room and the two furnished cage units in another room with similar housing conditions.

Microflora analysis : For each cage model and at each age tested (27 and 60 weeks of age), 20 batches of 3 eggs (only clean eggs for human food) have been analysed. These eggs have been randomly sorted from the total egg production of a day and represented about 10 to 20% of egg production of one cage unit. Each batch of 3 eggs have been placed in a sterile plastic bag and stored 24 h at 4°C. Thus, 200 ml of peptone water have been added and the eggs gently wrapped for 3 minutes (1 minute for each egg). The peptone solution was thus considered as the first dilution and used immediately for bacterial analysis. Total number of aerobic bacteria was estimated by

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surface plating of 0.1 ml sample on P.C.A. agar (AES ref. AEB 150702) and incubated 48 h at 30°C for colony counting. The results are expressed as the Log of the Colony Forming Units (CFU) per egg.

Egg quality : At 28 and 47 weeks of age, for each treatment, 100 - 120 eggs were randomly collected from the eggs laid on a two days period and weighted. Egg dimensions were measured to calculate eggshell index and thickness (Sauveur, 1988; Bain, 1990). Eggshell breaking strength was measured for each egg by Instron (UK527, Coronation Road, High Wycombe, UK). Then, eggshell weight was measured after breaking the eggs, washing the eggshells and drying at 105°C for four hours.

The estimation of broken eggs was done each day directly by visual examination. For cracked and dirty eggs, all the eggs laid in the week (tuesday to friday) were taken and pooled, for each system, in 6 replicates according to the location of the cages in the room. Detection was done using a small version of a commercial egg-candling machine.

Statistics : The results have been analysed using ANOVA with a factorial treatment structure comprising age and caging system treatments after Log transformation for bacterial counts, arcsin (square root of %) for cracked, broken and dirty eggs and without transformation of the data for the others egg quality parameters.

Results and discussion

Bacterial load : A slight but significantly lower bacterial count (table 1) was observed for all the caging systems at 60 weeks compared to 27 weeks. Protais *et al* (2003) have not observed any difference with age. In the present study, the lower bacterial count observed at 60 weeks is probably more a result of differences in environmental factors (effect of the season, summer for 27w and winter for 60w) than a direct effect of the age.

Whatever the age of the hens, a significantly higher bacterial count was observed for the eggs laid in furnished cages, except for C6B system at 27w that is especially high and similar at this date to the furnished cages from the same manufacturer. The differences observed are mainly due to the percentage of eggs laid outside the nest with a higher eggshell contamination (Mallet, unpublished data). A similar and higher difference have been previously observed by Protais *et al.* (2003) between conventional cages and aviaries.

Eggshell quality: As expected for the normal effect of age, an increase of egg weight and a decrease of egg breaking strength (table 2) as well as a higher number of cracked and broken eggs (table 3) were observed at 47w compared to 28w.

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With caging system, no significant differences were observed at any age regarding egg weight and eggshell solidity but we found that eggshell thickness was significantly higher in furnished cages ($p<0.05$) at 47w (Table 2). This may be due to a better feed assimilation as a consequence of a better welfare in furnished cages.
The number of cracked and broken eggs is globally significantly higher in furnished cages whatever the age (table 3) this can be a consequence of the presence of perches as previously observed by Tauson (1984). Nevertheless, this may be different from a model to another according to the disposition of furniture when available and to the effective surface available for the hens, especially for dirty eggs. For example if F15B cages with only 1 m^2 available gave really poor results regarding dirty eggs percentage compared to F15A (1.5 m^2), F15A gave similar or better results compared to C6B and C5A.

Table 1 : Evolution of mesophilic aerobic microflora. Log CFU/egg (mean ± standard deviation). Different letters are statistically different ($p < 0,05$).

Cage model	27 weeks	60 weeks
C.5A	4,31± 0,35 c	4,18 ± 0,26 c
C.6B	5,02 ± 0,17 a	4,08 ± 0,4 c
F.15A	4,61± 0,14 b	4,43 ± 0,3 b
F.15B	5,09 ± 0,19 a	4,95 ± 0,24 a
Cage model	p < 0,0001	
Age	p < 0,0001	
Interaction	p < 0,0001	

Table 2 : Evolution of egg weight and eggshell quality

Cage model	Egg wt. (g)		Eggshell					
			b. strength (N)		Index (g/100cm^2)		Thickness (mm)	
	28w.	47w.	28w.	47w.	28w.	47w.	28w.	47w.
C. 5A	63.3	66.7	38.5	34.9	8.67	8.74	0.369	0.372
C. 6B	62.7	67.6	38.5	35.3	8.64	8.75	0.368	0.372
F.15A	62.7	68.2	37.9	36.2	8.48	8.93	0.361	0.380
F.15B	62.8	68.9	39.3	36.5	8.79	8.81	0.374	0.375
Cage model	NS		NS		NS		NS	
Age	p<0.001		p<0.0001		p<0.001		p<0.001	
Interaction	NS		NS		p<0.05		p<0.05	

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Table 3 : Evolution of broken, cracked and dirty eggs.

		Cracked eggs (%)		Broken eggs (%)		Dirty eggs (%)	
	Age (weeks)	**28**	**47**	**28**	**47**	**28**	**47**
cage model	**C5A**	6.1	8.9	3.4	3.9	5.9	4.7
	C6B	3.3	8.2	1.4	1.9	5.6	4
	F15A	7	13.3	4.2	6.1	4.6	4.4
	F15B	11.2	20.1	3.1	4.7	7.2	6.4
P*-value	**cage model**	p <0.0001		p = 0.0002		p = 0.0123	
	Age	p <0.0001		p = 0.0294		NS	
	interaction	p = 0.0132		NS		NS	

*ANOVA results for repeated measures

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Session 11
Enrichment of egg for human nutrition

EGG ALLERGY-AN EMERGING ISSUE-

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Keywords : Food allergy, egg white, IgE antibodies, ovomucoid, ovalbumin, ovotransferrin, lysozyme

Abstract

Food allergy is an immune-mediated state of hypersensitivity resulting from exposure to food allergens and is most often mediated by immunoglobulin IgE. The number of patients suffering from food allergy has increased strikingly during recent decades. Food allergy is recognized as a significant public health problem. Approximately 2-3% of adults and 5-7% of children are plagued by food allergy. Egg allergy is one of the most frequently implicated causes of immediate food allergic reactions. The prevalence of egg allergy is about 35% of food allergic children. Elucidation of allergic reactions is more frequently cause by egg white than egg yolk. Egg white consists of 24 different protein fractions and a number of minor proteins. Among these proteins, ovomucoid (*Gad d1*), ovalbumin (*Gad d2*), ovotransferrin (*Gad d3*) and lysozyme (*Gad d4*) were identified as immune dominant proteins of egg white in egg-allergic patients. Detailed information about biochemical, immunological and structural properties of the relatively well characterized two major egg white allergens, ovomucoid and ovalbumin were give. Studies of B cell and T cell epitopes of both allergens were summarized.

Résumé

L'allergie alimentaire est un état d'hypersensibilité résultant de l'exposition à des allergènes et est souvent conduite par les immunoglobulines IgE. Le nombre de patients soufrant d'allergie alimentaire a fortement augmenté depuis quelques décades. L'allergie alimentaire est reconnue comme un problème de santé publique significatif. Environ 2-3% des adultes et 5-7% des enfants sont touchés par l'allergie alimentaire. L'allergie aux œufs est l'une des causes majeures dans les réactions allergiques

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alimentaires immédiates. La prévalence de l'allergie aux œufs est d'environ 35% chez les enfants présentant des allergies alimentaires. Les réactions allergiques aux œufs sont plus souvent causées par le blanc que par le jaune d'œuf. le blanc d'œuf consiste en 24 protéines majeurs et un grand nombre de protéines mineures. Parmi ces protéines, l'ovomucoïde (*Gad d1*), l'ovalbumine (*Gad d2*), l'ovotransferrine (*Gad d3*) et le lysozyme (*Gad d4*) sont identifiées comme des protéines immunes dominantes du blanc d'œuf chez les patients allergiques. Des informations détaillées sur les propriétés immunologiques et structurales de deux protéines majeurs du blanc relativement bien caractérisées (ovomucoïde et ovalbumine) sont données. Des études de cellules B et d'épitopes de cellules T des deux allergènes sont résumées.

Introduction

A food allergy is an adverse reaction to a food or food component that involves the body's immune system. Food allergy is recognized as a significant public health problem. It is estimated that 2-3% of adult and 5-7% of children suffer from food allergies which are defined for the purpose of this report type I IgE-mediated immunologic reactions to specific foods (Samson, 1990, Anderson, 1996). The number of patients suffering from food allergy has increased strikingly during recent decades (Emmett, 1996). The body's immune system recognizes an allergen in the food as a foreign antigen and produces antibodies to halt the invasion. As the battle rages, symptoms appear throughout the body. The most common sites are the mouth (swelling of the lips), digestive tract (stomach, vomiting, diarrhea), skin (hives, vases or eczema) and the airways (wheezing or breathing problems). IgE-mediated food allergies are associated with a wide variety of symptoms ranging from mild and annoying to severe and life threatening. It has revealed a list of more than 160 foods and food related substances that have been associated with allergic reactions in individuals. The general consensus in that the eight foods or food groups are the most common allergenic foods, more than 90% of all IgE-mediated food allergies on a world wide basis (Bousquet et al., 1998, FAO, 1995). These foods are milk, egg, fish, crustacean (shrimp, crab, lobster, crayfish), peanuts, soybeans, tree nuts and wheat. Many food allergens, particularly those most commonly associated with allergic reactions, are present as major protein components of the allergen. In general, allergenic proteins are comparatively stable proteins and this is a fact that proteins is much gain access to the immune system in an antigenically intact form to stimulate as allergic response (Taylor and Lehrer, 1996).

The type I allergic reaction is characterised by the production of allergen-specific IgE and the activation of mast cells. First, sensitisation by the allergen must occur. The allergen stimulates production of specific IgE antibodies. The specific IgE antibodies

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then attach to mast cells in various tissues and basophils in the blood. Mast cells and basophils contain granules that are loaded with physiologically active chemicals that mediate that allergic response (Church et at al., 1998). On subsequent exposure to the allergenic substance, the allergen cross-links two IgE antibodies on the surface of the mast cell or basophil membrane, stimulating the release into tissues and blood of a host of allergic response mediators such as histamine, one of the primary mediators responsible for many of the immediate symptoms that occur in IgE-mediated allergic reactions (Simons, 1998) (**Fig. 1**).

Egg allergy

Eggs from chickens (*Gallus domesticus*) are widely used for human consumption. Egg allergy is one of the most frequently implicated causes of immediate food allergic reactions. The prevalence of egg allergy is about 35% of food allergic children and children with atopic dermatitis, respectively (Crespo et al., 1995). The prevalence could be up to 45% in DBPCFC-positive children with atopic dermatitis (Sampson and Ho, 1997). Egg was the cause of anaphylactic shocks in 11.6% of case due to ingestion of foods (Monered-Vautrin and Kanny, 1995). Frequently, egg sensitivity disappears by the 4 or 4 years of life; however, one third of individuals have clinical sensitivity that lasts over 6 years (Crespo et al., 1995). Elucidation of allergic reactions is more frequently caused by egg white than egg yolk (Anet et al., 1985).

Major egg allergens

Eggs are composed of 56-61% egg white and 27-32% egg yolk. The egg white is approximately 87-89% water and 9-11% protein, whereas the egg yolk contains 50% water, 32-35% lipid and 16% protein (Li-Chan et al., 1995). The predominant protein in the egg white is ovalbumin, comprising 54% of the protein present. Other major proteins in the egg white are ovotransferrin (12%), ovomucoid (11%), ovomucin (3.5%) and lysozyme (3.4%). In addition these proteins, a variety of other proteins have been identified. Many previous studies have reported on the antigenicity and allergenicity of egg-white proteins; however, although ovomucoid, ovalbumin, ovotransferrin and lysozyme have been clearly identified as egg white allergens, the identification of the most important of these allergens remains a source of some scientific confusion and debate (**Table 1**). Miller and Campbell (1959) found, using skin tests, that the frequency of allergenic reaction to egg white had the following order: lysozyme > ovomucin > ovalbumin > ovomucoid. Bleumink and Young (1971)

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concluded that ovomucoid was the dominant allergen, and was more reactive than ovalbumin using a same method. Other groups reported that ovalbumin, ovomucoid, and ovotransferrin were the major allergens while lysozyme was a weak allergen (Hoffman, 1983, Langeland, 1982, Langeland and Harbitzm 1983), or that ovalbumin was the major allergen (Holen and Elsayed, 1990, Elsayed, 1993, Kahlert et al., 1992, Honma et al., 1996) using various electrophoretic and immunochemical techniques. Large amounts of IgE antibodies have also been detected in sera pooled from patients with egg allergy (Holen and Elsayed, 1990). Recently, ovomucoid has been reported to be the immuno-dominant protein of egg white in egg-allergic children (Bernhisel-Broadbent et al., 1994, Urisu et al., 1997, Cook and Sampson, 1997, Zhang and Mine, 1998, Zhang and Mine, 1999, Mine and Zhang, 2002) using sera from egg allergic patients. In spite of a large number of studies into egg-white allergy, no clear consensus has been reached as to relative antigenicity and allergenicity of egg-white proteins. Egg white consists of 24 different protein fractions and a number of minor proteins (Mine, 1995). The contradictory results on egg-white allergenicity may, therefore, be attributable to several aspects: (a) differing degrees of purity of individual protein fractions and antibody sources, (b) using sera from either humans with egg allergy or antibodies raised from experimental animals, or (c) different routes of administration (injection or oral administration). For example, commercially pure ovalbumin, which is generally accepted as the most allergenic protein and extensively studied as a model allergen, has been found to contain ovomucoid as an impurity which led to an overestimation of the dominance of ovalbumin as the major egg-white allergen (Bernhisel-Broadbent et al., 1994, Mine and Zhang, 2001). These antibodies were raised in the laboratory through immunisation of the animals by the injection of emulsified antigen, whereas specific antibodies from allergic patients were sensitised by antigens absorbed from the gastrointestinal tract. It is unclear whether antibody specificity is influenced by the different administration routes used for antigen sensitisation. The immuno-dominant epitopes for IgG responses in animals from injection may differ from IgE responses in humans from ingestion of egg.

The binding activities of IgG and IgE antibodies from egg-allergy patients to physically or chemically treated egg-white proteins were examined and compared with those of rabbit anti-egg white IgG antibodies (Mine and Zhang, 2002). The sera from eight patients and four rabbit antibodies were used. The binding activities of human IgG antibody to partially denatured ovotransferrin, ovalbumin, and lysozyme forms were increased, while carboxymethylation (RCM) and heat treatment caused a dramatic decrease in the antigenicity of ovotransferrin and ovomucoid. Ovotransferrin and ovomucoid were major immunogenic antigens for the rabbit IgG response. Urea also caused ovotransferrin to exhibit greater rabbit IgG binding activity. In contrast, human and rabbit antibodies did not react with ovomucin. Partially denatured ovotransferrin and lysozyme also induced strong IgE binding activities. The allergenicity of ovotransferrin, ovomucoid, and lysozyme were decreased by RCM,

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whereas ovalbumin retained its binding capacity. These results suggested that anti-ovalbumin IgE recognises more sequential epitopes, and anti-ovomucoid and lysozyme antibodies recognise both conformational and sequential epitopes.

Biochemical, immunological and structural properties of major egg allergens.

Ovomucoid: Ovomucoid (*Gal d1*) is the dominant allergen found in egg white. Ovomucoid is a glycoprotein with a molecular weight of 28 kDa (Kato et al., 1987), and it has a pI of 4.0 and is a trypsin inhibitor (Kato et al., 1987). Being a dominant allergen it has prominent carbohydrated domains (Matsuda et al., 1982), but it has been demonstrated that the carbohydrate moieties have no impact on the IgE binding of ovomucoid (Besler et al., 1997). On the other hand, the importance of the carbohydrate epitope in N-glycosylated domain III (N175) to allergic reactions to egg white has been documented (Matsuda et al., 1985), while it has been shown that the carbohydrate region in domain III inhibits the effect of IgE binding activities (Zhang and Mine, 1998).

Epitopes are dependent on either the tertiary structure of the protein (conformational epitope) or several amino acid sequences on the protein surface (linear epitope). Previous studies have suggested that the allergenicity and antigenicity of ovomucoid are quite resistant to enzyme digestions, heat and denaturation (Kurisaki et al., 1981; Matsuda et al., 1985; Nolan-Kovacs et al., 2000)), suggesting that antibody binding is directed towards a linear epitope. It has shown that the major epitopes on each domain primarily had a linear structure, and that the IgG and IgE binding activities were more dominant to the third domain (Zhang and Mine, 1998). Each domain has common epitope(s) (homology) which are recognised by peripheral blood T cells from allergic patients (Bock and Atkins, 1990).

Recently the entire epitope sequence binding to specific IgG and IgE patients' sera in the whole ovomucoid molecule comprising all the three domains have been elucidated by several groups. Pooled patients' sera identified three main IgE binding epitopes in domain I (amino acids 32–42, 40–50 and 56–66), five IgE binding epitopes (amino acids 71–75, 80–90, 101–105 and 121–130) in domain II and two major regions (animo acis 159–174 and 179–186) in domain III. Pooled patients' sera recognised three IgG epitopes on domain I (amino acids 6–15, 31–35 and 46–50), two on domain II (amino acids 71–75 and 101–105) and three on domain III (amino acids 160–167, 165–174 and 177–183). Among these epitopes, three IgE epitopes (amino acids 56–66, 80–90 and 121–130) and one IgG epitope (6–15) were recognised by only either the IgE or the IgG antibody (Mine and Zhang, 2002) (**Fig**. **2**). Cooke and Sampson (1997) identified five IgE epitopes (amino acids 1–20, 49–56, 85–96, 115–122 and 175–186) and seven IgG epitopes (amino acids 3–11, 23–56, 69–74, 87–104, 119–124, 138–149

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and 175–186) using the SPOTs membrane to probe the sera of seven pooled egg-allergic patients. Two distinct IgE epitopes (amino acids 90–121 and 134–186) were determined by trypsin cleavage of ovomucoid and subsequent immunoblot probing with egg-allergic patients' sera (Besler et al., 1999). More recently, Holen et al. (2001) reported one novel IgE epitope (amino acid 61–74) and another common IgE and IgG epitope (amino acid 101–114) using synthetic overlapping peptides. In addition to these two epitopes, four epitopes (amino acids 41–56, 71–84, 131–144 and 171–186) were identified as exclusively T-cell epitopes with no affinity to specific antibodies (Holen et al., 2001).

Ovalbumin: Ovalbumin (*Gad d2*) is a major allergen in hen egg white consisting of 385 amino acids with a molecular weight of 45 kDa (Nisbet *et al*., 1981). It has been reported that treatment of ovalbumin by carboxymethylation, 6M urea or heating at 95°C had no significant effect on its binding capacity to human IgE to ovalbumin derived from egg allergy patients' sera (Mine and Zhang, 2002). This indicates that anti-obalbumin IgE antibodies from egg allergic patients recognise mainly sequential epitopes. The IgE class of antibodies for ovalbumin also have been shown to exist in sera from patients with allergy (May *et al*., 1977). Epitope mapping of specific regions in ovalbumin have been done (Elsayed and Stavseng, 1994; Masuda *et al*., 2000) by several groups using enzyme digested fragments and synthetic peptides. The studies on IgE antibody responses to ovalbumin have been shown earlier (Elsayed *et al*., 1991; Kahlert *et al*., 1992). Protein structure also plays a critical role in determining the immunodominant IgE-binding epitopes and there seems to be a link between the allergen structure and the IgE-binding epitopes (Shimojo *et al*., 1994; Sen *et al*., 2002).

Recently, we have mapped five distinct IgE recognition sites (residues 38-49, 95-102, 191-200, 243-248 and 251-260) were identified in the primary sequence of the ovalbumin protein by the pooled sera obtained from 18 patients with egg hypersensitivity and these immuno dominant epitopes seem to be distributed throughout the ovalbumin primary sequence (Mine and Rupa, 2003). There have been several reports on IgE binding sites of ovalbumin using synthetic peptides or chemical cleavage. It has been reported that the IgE epitopes on ovalbumin recognised by human IgE antibodies in patients with egg allergy using synthetic peptides or cyanogen bromide-cleaved fragments by means of Western blots and 2D-PAGE quantitative precipitation inhibition assay. The amino acid residue 1-10 (Elsayed et al, 1988), 323-339 (Johnsen and Elsayed, 1990), 34-46, 47-55 (Elsayed and Stavseng, 1994), 41-172 and 301-385 (Kahlert et al, 1992). Furthermore, detailed IgE epitope mapping in the C-terminal region comprising residues 347-385 of ovalbumin was carried out using immunoblot and histamine release assays. The amino acid residues 347-366, 347-385, 357-366, 357-376 and 367-385 were identified as allergenic epitopes of ovalbumin (Honma et al., 1996). Our recent sequential IgE epitope mapping results seem to have totally different compared to earlier works. We could

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not identify any IgE binding sites in N- or C-terminal regions where previous workers have reported in regions of 1-19 and 347-385. The differences of these results could be attribute to the patient populations employed in each study. Elsayed and Stavseng (1994) selected 3 of 16 patients' sera due to their total IgE and RAST class and pooled sera were used. It seems that they used only selected serum. Furthermore, the earlier works (Elsayed et al, 1988; Kahlert et al, 1992) did not state enough information about patients sera. Three patients who showed RAST scores for egg white classes 3 were used for C-terminal IgE binding site mapping (Honma et al, 1996). Therefore, there have been limits the general applicability of the early work to compare the present results which were obtained from 3 pooled groups of 18 patients sera with egg allergy. Further investigation using a large number of patient sera could provide more reliable information to address this conflict.

The location of each of IgE sequential epitopes obtained in our study in tertiary structure of ovalbumin was analysed (**Fig. 3**). As expected, four of five epitopes in three-dimensional structure are exposed to protein surface except an epitope 191-200 and the major secondary structure is consisting of β-sheet and β-turn. 38-49 is part to a pair of α-helix A (39-44) and β-turn (45-48), and the following of 191-200; β-sheet s3A (191-196)+random (197-200), 243-248; β-turn (type II) (243-248) and 251-260; β-sheet s3B (251-255)+β-turn (type I) (256-258), respectively. One epitope 95-102 is comprised of a single α-helix D (94-104) (Stein et al., 1991). These results indicate that major structural components of IgE epitopes in ovalbumin are β-sheet and β-turn structures (Rupa and Mine, 2003).

Ovotransferrin: Ovotransferrin (*Gal d 3*) has a molecular weight of 77 kDa, and pI of 6.0. It is 686 amino acid residues and sequence have been identified (Williams, et al., 1982). It consists of 12 disulfide bonds and contains 2.6% carbohydrate. It has antimicrobial activity and iron-binding properties. Ovotransferrin has also been documented in several studies as a major egg allergen (Langeland, 1983a, b). However, its epitopes and biochemical characterisation have not been conducted yet.

Lysozyme: Lysozyme (*Gad d4*) is a 14.3 kDa protein with a pI of 10.7. Its 129 amino acids have been sequenced. It is a single polypeptide chain cross-linked by four disulfide bridges (Canfield, 1963). The chain is folded upon itself so that the first 40 residues from the N-terminal end form a compact globular domain. There is a second hydrophilic domain (residue 40-85) that forms one site of the active cleft. The role of lysozyme in egg allergy has not been established.

Molecular approaches to the study of egg allergens.

Recombinant DNA technology is becoming more commonly used for producing allergenic proteins *in vitro*. During the last ten years, in addition to molecular

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approaches to the immunological and structural studies of allergenic proteins, many recombinant allergens have been produced that offer important new tools for the diagnosis and therapy of allergies. Recombinant proteins can be produced in large quantities and with great purity, making it easier to study the molecular design of the protein when attempting to reduce the allergenic structure by genetic engineering. To date, more than 40 food allergens from fruits, vegetables, nuts, milk, and seafood have been expressed as recombinant proteins (Lorenz et al., 2001). Studies have been done on the third domain of ovomucoid on its protease inhibitory activity using site specific mutagenesis (Kojima et al., 1999). However, recombinant proteins are in general not identical copies of their respective native analogue and they may have slight modification in their structures. Therefore, it is a critical step to investigate the immunological comparison of native and recombinant allergens before subjected to further studies. The ovomucoid third domain (DIII) cDNA was sublconed into pGEMT-vector and the resultant plasmid (pGEMDIII) was inserted into a pGEM-4T-2 glutathione-S-transferase (GST) fusion vector. The GST-DIII fusion protein was expressed in *Escherichia coli*. Measurement of circular dichroism spectra indicated that the recombinant third domain (DIII) had a structure that was slightly less compact than that of the native form. Immunoblot analysis showed that the human IgE binding activity of DIII was identical to that of native DIII, while its activity was significantly increased to IgE antibodies from egg-allergic patients when tested with an enzyme-linked immunosorbent assay. These results indicate that recombinant DIII has similar sequential epitopes, but may have more predominant conformational epitopes than native analogues (Sasaki and Mine., 2001).

Five genetically modified ovomucoid third domain (DIII) mutants which were substituted single or double amino acids within its IgE and IgE epitopes, were prepared and compared their antigenicity and allergenicity with native analogue using western immunoblot and enzyme-linked immunosorbent assay. Two site mutations of glycine at 32 and phenylalanine at 37 positions with methionine and alanine caused almost complete loss of IgG and IgE binding activities of human sera derived from egg allergic patients as well as disruption of α-helix structure which comprise of part of IgG and IgE epitopes. This indicated that glycine 32 and phenylalanine 37 have an important role on its antigenicity and allergenicity as well as structural integrity of ovomucoid DIII (Mine et al., 2003).

A gene coding for the cDNA representing an entire ovomucoid or ovalbumin molecule has been cloned in *Escherichia coli* under the control of T5 promoter fused with six-Histidine tag at the amino terminal end. Upon induction, the *E. coli* cells harbouring this construct, expressed the recombinant protein as a soluble fraction and the recombinant ovomucoid and ovalbumin protein were purified. Immunoblot analysis showed that human IgE and IgG binding activities of the recombinant ovomucoid or ovalbumin was identical to that of native analogue. The recombinant ovomucoid and

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ovalbumin constructed in our lab could be used for further studies on the biochemical, immunological and structural studies of egg allergens (Rupa and Mine, 2003a, b).

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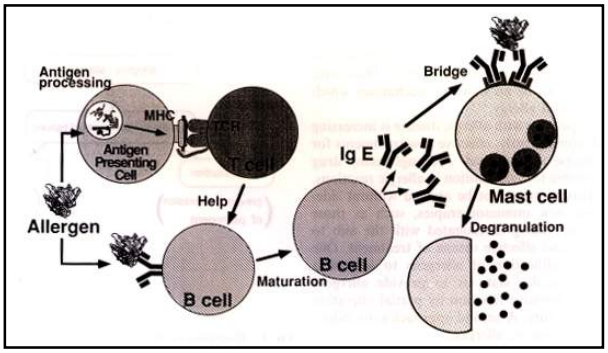

Fig. 1. Mechanism for type I allergy (adapted from Biosci. Biotech. Biochem., 60: 1749-1756, 1996).

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Table 1. Characteristics of major egg allergens.

Allergens	% of albumen Protein	M.W. (kDa)	pI	Chracteristics
Ovalbumin	54	45	4.5	phosphoglycoprotein
Ovomucoid	11	28	4.1	Inhibits trypsin
Ovotransferrin	12-13	76	6.0	Binds metallic ions
Lysozyme	3.4	14.3	10.7	Lyzes some bacteria

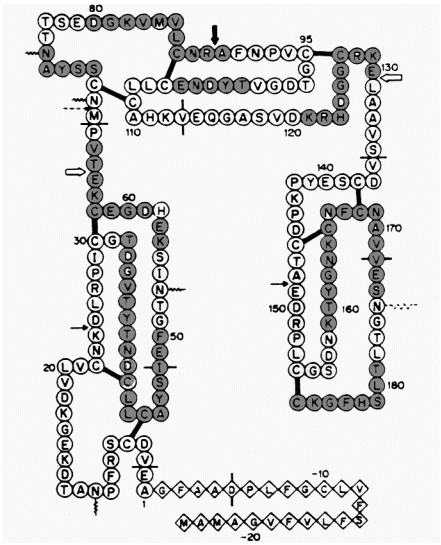

Fig. 2. Schematic representation of IgE epitopes in ovomucoid. The shaded amino acid sequences are shown each epitope (adapted from Biochem. Biophys. Res. Comm., 292: 1070-1074, 2002)

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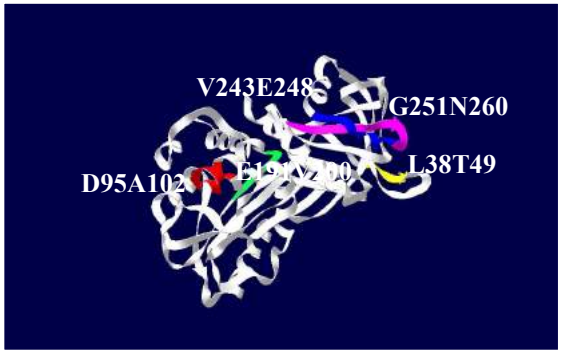

Fig. 3. Location of IgE epitopes in ovalbumin. (Adapted from Protein Eng., 2003 in press).

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PRODUCTION OF HEALTH PROMOTING FUNCTIONAL EGGS

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Keywords : Functional eggs, Herbal extracts, Cholesterol, Omega-3 fatty acids, Anti-oxidants

Abstract

Inspite of high nutritional value of the egg and mounting evidences showing no significant correlation between dietary and serum cholesterol levels, some people are still restricting the consumption of cholesterol rich foods like eggs. To overcome this problem, scientists have developed low cholesterol and omega-3 (O-3) fatty acids rich functional eggs. Now the researcher in designer egg production is aiming for utilising the egg as a carrier for several health promoting components like, multiple anti-oxidants, chelated minerals, vitamins and herbal extracts to reduce LDL cholesterol, atherosclerosis, cancer, arthritis, hypertension, diabetes and gastric ulcers and for immunomodulation. Ultimately such health promoting functional eggs will serve both as an excellent food as well as a safe medicine for several chronic diseases.

Resumé

Malgré la haute valeur nutritionnelle de l'œuf et les travaux scientifiques ne montrant aucune corrélation significative entre la consommation d'œuf et les niveaux de cholestérol sanguin, la communauté médicale continue de déconseiller la consommation d'aliments riches en cholestérol, comme les œufs. Afin de surmonter ce problème, les scientifiques ont développé des œufs appauvris en cholestérol et riches en acides gras fonctionnels omega-3 (O-3). Actuellement, les chercheurs envisagent d'utiliser l'œuf comme un vecteur de composés antioxidants, de minéraux chélatés, de vitamines, et d'extraits d'herbes pour réduire le cholestérol, l'athérosclérose, certains cancers, l'arthrite, l'hypertension, le diabète et les ulcères gastriques et pour immunomodulation. Finalement de tels œufs fonctionnels pourraient servir comme une aliment et en même temps de médicament sûr pour plusieurs maladies chroniques.

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Introduction

In order to increase egg consumption, it is essential to make it more safer, nutritious and healthy. As early as 1934, Cruickshank has discovered that the fatty acid composition of the egg yolk can be altered by diet. However, this discovery was not utilised until the health promoting properties of omega-3 fatty acids (O-3 FA) had been revealed by Simopoulos (1991). Based on these findings, Sim (1993) has incorporated O-3 FA in egg yolk at high levels, by feeding hens with flaxseeds amd camala and patented it as the "Dr.Sim's Canadian Designer Eggs". Later, Farrell *et al.* (1994) produced O-3 FA rich eggs by feeding hens with various fish and vegetable oils rich in O-3 FA. Since O-3 FA rich foods will under go rancidity quickly, natural anti-oxidants like vitamin-E and organic selenium are added to the egg, by feeding them to hens. Subsequently Van Elswyk (1997), Sujatha, (2002), Surai, (2002), Narahari *et al.* (2003) and Esmail (2003) have improved the "designer egg production technology", by incorporating several health promoting components in the egg.

Few initial experiments in designer egg production had resulted in lowered egg production and undesirable flavours, but these defects were rectified subsequently by adding anti-oxidants, using multiple sources of O-3 FA and by improved managemental practices.

Van Elswyk (1997) used flax seed, fish oil or marine algae in hen's diet for incorporation of O-3 FA in their eggs. At 15-30 g/kg level, fish oil was very effective in raising the yolk O-3 FA levels; but it imparted an undesirable flavour to the egg. Flax seed influenced the yolk O-3 FA levels only at 150g/ kg level in the hen's diet. The marine algae proved to be very efficient not only in raising the O-3 FA content of egg yolk; but also the yolk carotenoid pigments.

Diet eggs (2003), Gold circle farms eggs (2003) and many other commercial layer farms are producing designer eggs by supplementing flaxseed, fish oil, canola oil, vitamin E and organic selenium (Sel-plex). Such eggs are having high levels of O-3 FA, with a O-6: O-3 FA ratio around 1.0, compared to more than 12 in ordinary eggs.

Surai *et al.* (2000) had enriched the designer eggs by supplementing vitamin E, lutein and selenium to the hen's diet. Feeding such enriched eggs had lowered the serum LDL-C and platelet aggregation in humans. Surai and Dvorska (2001) incorporated organic selenium in layer diets at 0.2, 0.4 or 0.8 mg Se/ kg. At 0.2 mg se/ kg diet se in yolk and white was increased by 2 and 3.8-fold, respectively; whereas at 0.8 mg level, the increase was 3.7 and 12.3-fold, respectively

Usharani (1997) enriched the hens feed with either 10g/ kg basil *(ocimum sanctum)* leaves or 750 mg/ kg nicotinic acid, to reduce the egg and serum cholesterol levels.

Narahari (2001 and 2003) suggested various feed supplements to be incorporated in the hen's diet (Table 1) to produce the health promoting functional eggs. All these

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supplements are not needed at a time. Only few of them, based on the local availability, cost and the purpose have to be selected.
Sujatha (2002) fed hens with one control and three special diets containing flaxseed, Anchovy fish oil, vitamin E, ethoxyquin, organic selenium (Sel-plex) and spirulina, to produce eggs low in cholesterol and enriched with O-3 FA, vitamin E, selenium and carotenoid pigments.

Table 1. Feed supplements needed to produce health promoting functional eggs.

Feed supplement	***Quantity/ tonne of feed***
Fatty fish meal	50 - 100 kg
Fish oil (Stabilised or microencapsulated)	10 - 25 kg
Flax seeds	100 - 150 kg
Rapeseed/ canola oil	5 - 10 kg
Corn glutten meal, Azolla	50 - 100 kg
Alfalfa meal	10 20 kg
Marigold petal meal	3 - 5 kg
Grape seed meal	5 - 10 kg
Tomato pomace	5 - 10 kg
Glandless cottonseed protein	10 - 30 kg
Garlic powder	1 - 3 kg
Turmeric power	0.15 - 1kg
Capsicum or red pepper powder	1 - 2 kg
Basil leaves	1 - 3 kg
Spirulina	0.5 - 1 kg
Activated charcoal	1 - 2 kg
Anti-oxidant	100 - 250 g
Vitamin E	100 - 400 g
Organic selenium (Sel-plex)	100 - 200 g
Marine algae	5 - 10 kg
B-complex vitamins	Double the recommended levels
Trace minerals	1.5 times recommended levels
Herbs	2 - 5 kg
Organic chromium (Bio-chrome)	100 - 200 g

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Results and discussion

Based on the work done earlier by many workers, the role of different feed supplements in hen's diet on functional egg composition and quality are reported in Table 2.

Table 2. Role of feed supplements on function egg production

Feed supplements	*Functions*
1. Flaxseed, canola oil, fish oil, marine algae, pearl millet	Increase O-3 FA level in yolk, to bring O-6: O-3 FA ratio to 1:1
2. Spirulina, marigold petal meal, alfalfa, corn glutten meal, Red pepper, carotenoid pigments, Azolla	Improves yolk pigmentation, anti- oxidants anti-carcinogenic
3. Vitamin E organic selenium, turmeric powder and anti-oxidants	Prevent oxidation of PUFA, free radicals & off flavours, and immunomodulation
4. Nicotinic acid, pectins, plant sterols, spirulina, garlic, Basil levels	Reduce yolk and serum cholesterol levels
5. Organic chromium, fenugreek, curry leaf & other herbs rich in saponins and certain alkaloids	Stimulates insulin secretion in humans consuming such eggs
6. Spices and Herbs having active principles like Eugenol, tocotrienols, chelated minerals, allycin, lycopene, nirangenin, sulforaphane, statin flavinoid compounds etc. like *solanvm nigrum, yeast, eclipta elba, phylianthus niruri*	Immunomodulation, reduces hypertension, cancer, atherosclorosis & liver disorders

Table 3. Effect of diet on serum lipid profile (Usharani, 1997)

Trait	*Control*	*750mg/ kg nicotinic acid*	*10g/ kg Basil leaves*
Total serum cholesterol (mg %)	$238^{a} \pm 9.4$	$124^{b} \pm 6.8$	$109^{c} \pm 4.0$
HDL - C (mg %)	$27^{a} \pm 3.4$	$120^{c} \pm 6.1$	$114^{b} \pm 4.8$
LDL - C (mg %)	$95^{a} \pm 2.1$	$38^{b} \pm 1.3$	$38^{b} \pm 3.0$
VLDL - C (mg %)	$326^{a} \pm 1.1$	$45^{c} \pm 1.2$	$49^{b} \pm 1.1$
Triglycerides (mg %)	$479^{a} \pm 20.1$	$189^{b} \pm 7.7$	$193^{b} \pm 5.8$

Means within each row bearing different superscripts differ significantly ($P<0.01$)

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Table 4.Composition of egg of hens fed 3 different functional feed (Sujatha, 2002)

Traits	***Control***	***Flaxseed + E + Spirulina***	***Fish oil + org. Se + Spirulina***	***All 5 supplements***
Yolk carotenoid pigments (µg/g)**	$24.5^{a} \pm 0.12$	$65.1^{b} \pm 0.13$	$65.8^{b} \pm 0.17$	$65.0^{b} \pm 0.14$
Yolk cholesterol (mg/g) **	$12.9^{a} \pm 0.13$	$9.9^{b} \pm 0.19$	$9.5^{b} \pm 0.12$	$10.0^{b} \pm 0.19$
O-3 FA g/100g TFA				
C18: 3**	$0.16^{a} \pm 0.02$	$7.57^{d} \pm 0.24$	$0.39^{b} \pm 0.03$	$6.04^{c} \pm 0.16$
C20:5n-3**	$0.06^{a} \pm 0.01$	$0.35^{b} \pm 0.02$	$0.61^{d} \pm 0.04$	$0.48^{c} \pm 0.02$
C22:6n-3**	$0.17^{a} \pm 0.01$	$2.70^{b} \pm 0.17$	$6.81^{d} \pm 0.20$	$3.69^{c} \pm 0.10$
Vit.E (µg/g yolk)**	$97^{a} \pm 3.8$	$297^{b} \pm 7.80$	$170^{b} \pm 6.1$	$238^{c} \pm 4.7$
Se in yolk (ng/g)**	$188^{a} \pm 8.3$	$178^{a} \pm 3.8$	$420^{c} \pm 6.3$	$356^{b} \pm 5.2$
Se in white (ng/ g)**	$51^{a} \pm 1.7$	$46^{a} \pm 3.5$	$99^{c} \pm 3.2$	$86^{b} \pm 1.7$

** Means within each trait bearing different superscripts differ significantly (P<0.01)

Table 5. Effect of spirulina and basil leaves on lipid profile (Narahari *et al., 2003)*

Trait	*Control feed*	*Functional feed (FF)*	*FF + 2g/ kg spirulina*	*FF + 2g/ kg Basil*	*FF + S + T*
Carotenoid pigments (µg/g)**	24.5^{a}	36.8^{b}	56.1^{c}	44.8^{b}	65.0^{c}
Cholesterol (mg/g)**	12.9^{a}	11.4^{b}	9.9^{c}	9.5^{c}	9.1^{c}
O-3 FA (g/100g TFA)**					
C18:3	0.16^{a}	5.9^{b}	6.1^{b}	5.7^{b}	6.2^{b}
C20:5	0.06^{a}	0.58^{b}	0.61^{b}	0.60^{b}	0.63^{b}
C22:6	0.17^{a}	4.6	5.1	5.1	5.3
Vit. E (µg/g)	82.8^{a}	290.5^{b}	301.2^{b}	292.4^{b}	303.3^{b}
Serum cholesterol (mg/dl)**					
T-C	177.4^{a}	164.7^{b}	145.2^{c}	140.0^{c}	130.9^{c}
VLDL-C	115.9^{a}	97.5^{b}	76.9^{c}	69.5^{c}	61.7^{c}
LDL-C	30.4^{a}	24.8^{b}	20.5^{bc}	21.0^{bc}	19.0^{c}
HDL-C	31.1^{a}	42.4^{b}	47.8^{bc}	49.5^{c}	50.2^{c}
Trigly -cerides	841^{a}	760^{b}	719^{c}	720^{c}	708^{c}

All these experiments have proved that the designer egg composition and quality can be further improved by supplementing several health promoting herbs, chelated minerals and vitamins. However, the real health promoting and therapeutic uses of such functional eggs have to be studied in humans, involving large number of volunteers.

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CONTENTS

NUTRITIONAL PROPERTIES of OSTRICH EGG

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Keywords : Ostrich egg, nutrition, nutrition properties, composition

Abstract

In this research, nutritional properties of ostrich (Struthio camelus) egg will be mentioned. Ostrich eggs are obtained from a ostrich farm in Bursa. At the end of the analytic and sensory analysis, it is determined similar properties between hen eggs and ostrich eggs except quantity. At the end of the analysis, it is determined that the ostrich eggs are an alternative to hen eggs because of nutritional value. Moreover, with this study it is aimed to introduce the ostrich eggs to the breeder and the consumer.

résumé

Dans cette étude, les caractéristiques physicochimiques et les propriétés fonctionnelles des œufs d'autruche ont été analysées. Les œufs d'autruche provenaient d'une ferme de Bursa. Hormis la quantité très largement en faveur de l'œuf d'autruche, les données analytiques et sensorielles obtenues n'étaient par notablement différentes de celles de l'œuf de poule. En raison de sa valeur nutritionnelle, l'œuf d'autruche peut constituer une alternative à l'œuf de poule. Parallèlement, cette étude avait pour mission de mieux faire connaître l'œuf d'autruche aux sélectionneurs et consommateurs.

Introduction

Having been stared in South Africa, in 1860s, ostrich breeding has been developing all around the world, especially in Europe and America (Alkýn 2001).
Ostriches (*Struthio camelus*), being the biggest member of the Ratite family (Alkin 2001), start laying eggs when they are 18 – 24 months old and depending on the nutrition they take, life conditions and climate, they lay eggs between July and November in Southern Hemisphere and between March and September in Northern

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Hemisphere (Sell 1993). Domestic ostriches generally reach sexual maturity when they are 2 years old. Egg production starts within 5 – 10 days after the first mating session and an ostrich lays 10 – 30 eggs for the first year. Towards the 5^{th} and 6^{th} years, production efficiency increases and an ostrich can lay 40 – 80 eggs with proper care and feeding (Anonymous 2001). Eggs in the beginning of the eggs laying session being unfertilised, are used as food and after emptied, as decorative commodities like lamps or teapots (Schuler 1999).

The fresh egg should be boiled for 1 hour for being soft boiled and for 1.5 hours for hard boiled. Under the conditions of our country, eggs laying session starts in spring and ends in fall (Anonymous 2001).

It tastes like goose egg and the best soufflé of North Africa is cooked from ostrich eggs. It can be kept in refrigerator for 15 days to be used in cases needed (Osgrow 1999). Ullrey and Allen state that ostrich eggs taste like turkey eggs.

Material and methods

Material

The ostrich eggs are obtained from a special ostrich farm in Bursa, brought to the lab daily and the content has been kept in refrigerator during the period of analysis. Weight, width, height, shell thickness, white/yolk ratio, dry matter, ash, protein, fat and total sugar analysis's were carried out on the eggs. In addition to that, ostrich egg omelette and chicken egg omelette has been subject to sensorial analysis by 60 persons about colour, taste and smell.

Methods

Egg Weight

Eggs were weighed by a precision balance with 0.01 g precision (Yücel 2000).

Egg Width and Height

The height and width of the eggs were measured by a compass and the results were given in mm. (Yücel 2000).

Egg Shape

Egg shape was obtained by dividing egg width by egg length and than multiplying by 100 (Yücel 2000).

White and Yolk Ratio

After the eggs were beaten, the white and yolk ratio was obtained by weighing with 0.01 g precision and the results were stated as white/yolk ratio (Yannakopoulos and Tserveni – Gousi 1986).

Shell Proportion

The eggs emptied, were weighed with 0.01 gram precision and the results were stated as g (Yannakopoulos and Tserveni – Gousi 1986).

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Smell
Sensorial smell analysis was carried out on the samples (Yalýn 1997).
Density Determination
100 mL egg was weighed and density was obtained by comparing the result with the volume.
pH Determination
pH analysis was carried out in 20^0C temperature by using a pH meter (Ýnal 1992).
Total Dry Matter Determination
The samples were made homogenous and dried in 105^0C temperature until they reach fixed weight and the results have been stated as g/100 g (Yücel 1992).
Moisture Determination
The moisture estimations of the samples were obtained by subtracting the dry matter quantity from 100 (Yalýn 1997).
Protein Determination
The protein ratio of the samples that are made homogenous was obtained by using Kjeldahl method and the results were stated as g/100 g (Yýldýrým 1988).
Fat Determination
The fat ratio of the samples was obtained by using Folch method and the results were stated as g/100 (Harris and mates 1994)
Ash Determination
Samples were weighed in panniers with known tare than they were heated in ash furnace with 550^0C temperature. The results were stated as g/100 g. (Vural and Öztan 1996).
Sensorial Analysis
Omelette was cooked from chicken and ostrich egg and was evaluated by 40 persons about colour, taste and smell.

Result and discussion

An ostrich egg is equivalent to 24 chicken eggs as it can be seen in Figure 1 (Woodard 2001). In the analysis carried out, the average weight was calculated as 14228 g Ýnal in 1992 stated that, a chicken egg is about 50 – 60 g.

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Figure 1. Chicken egg (on the left) and ostrich egg (on the right)

As seen in figure 2, the content of chicken egg is too less when compared to the content of an ostrich egg. Yücel (2000), states that, white quantity of a chicken egg is 59,29 %, yolk quantity is 28,54 % and shell quantity is 12,17 %. Kocakaya (2000) states that, yolk of an ostrich egg, constitutes 1/3 of the total. As seen in table 1, 22.05 % of an ostrich egg is yolk, 59.69 % is white and 18.26 % is shell. Apart from the physical characteristics, the ostrich egg is similar to chicken egg.

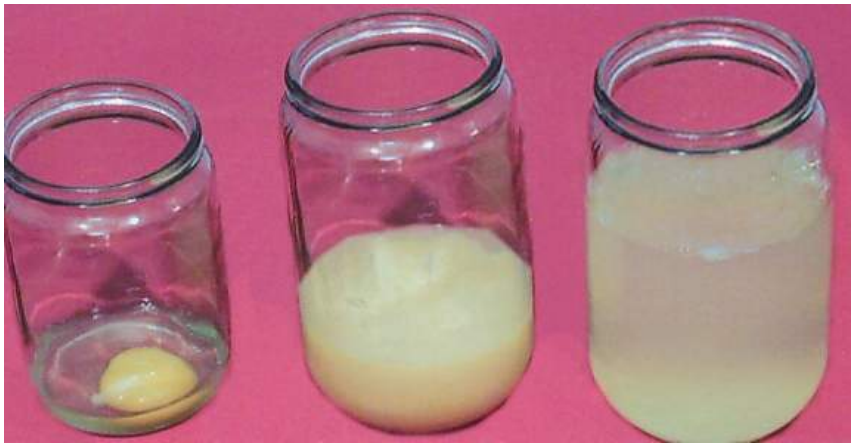

Figure 2. Chicken egg (on the left pot) and ostrich egg (on the right pot) composition

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The results of average analytic analysis are stated in Table 1 and Table 2.

Table 1. Physical properties of ostrich egg

Analysis name	**Results**
Weight (g)	1428
Egg Widht (cm)	14
Egg Height (cm)	16
Egg Shape (%)	87.50
White/Yolk Ratio	2.70
Shell ratio (%)	0.25
White ratio (%)	22.05
Yolk ratio (%)	28.54
Shell thick (mm)	0.32

Many researches stated that the weight of an ostrich egg can vary between 1200 – 2000 g. Narahari and his colleagues (2000) stated that, it varies between 700 – 1500 g. İpek and Şahan (2001) mentioned that, an average ostrich egg weighs 1444 g. Average egg weight was found to be 1428 g in this research. The results obtained in these researches are compatible with the datum collected by other researchers. Yücel (2000) states that, the most suitable shape index for chicken egg is 71 – 77. According to this, the shape index of the ostrich egg is higher than the shape index of a chicken egg.

Table 2. The analysis of ostrich egg white and ostrich egg yolk.

Analysis name	**Results**	
	Egg white	**Egg yolk**
Smell	Yok	Var
pH	8.37	6.81
Total drymatter (%)	10.05	45.15
Moisture (%)	89.95	58.85
Protein (%)	8.11	14.01
Fat (%)	20.48	0.41
Ash (%)	1.63	0.48
Density (g/cm^3)	1.0319	1.0466

As seen in Table 2, the ostrich egg is rich in nutrition. İnal (1992) indicates that, 87.2% of egg white is moisture, 10.3% is protein, 0.6% is ash and in yolk; 46.1% is moisture, 16.5% protein, 34.9% is fat and 1.3% is ash. Narahari (2001) states that, chicken egg contains 13% protein with high biological value as well. According to this, ostrich egg contains lesser fat and similar quantity of protein an mineral when compared to chicken egg. Kocakaya (2000) remarks that, since the ostrich egg contains less cholesterol and is lean and scentless, this contributes to its diet properties. Similar findings were obtained in this research as well and it is found that, ostrich egg

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is more valuable as a food when compared to chicken egg. Because, consuming foods with lesser fat, is advised by all dieticians. This characteristics of ostrich egg, contributes to the value of it.

In sensorial analysis, not much difference was found between the omelets cooked from ostrich and chicken egg. Participants could not distinguish one egg to the other and no significant difference was obtained from the taste point of view. Most of the participants mentioned about visual differences like color and appearance. This is thought to be because ostrich egg yolk is lighter in color. Because, the omelet cooked from ostrich egg looks whiter. This is considered as detrimental from the view of the customers. Only 3 % of the participants found the taste of the omelet (ostrich egg) distasteful. On the other hand, 70% of the participants claimed that, they will prefer omelet cooked from ostrich egg, because it is softer, lighter and it dissolves in mouth easily.

In the researches carried out, it is found that the yolk of ostrich egg has a smell special to itself. But this smell was not sensed by the participants who tested the omelet. This is thought to be because of the high white/yolk ratio of the ostrich egg.

Nearly 700 ostrich farms and 7000 – 9000 ostriches are found in our country. However, almost 500 pieces of ostrich eggs sold as decorative commodity, since they are small and some farms do not have an incubation machine. This situation is considered as a loss for our country which needs more animal food.

Kocakaya (2000) states that any meal cooked from chicken egg can also be cooked from ostrich egg. Ostrich egg was used as an ingredient in this research and the participants could not distinguish it. In addition, it was used as an ingredient for baking cake and the same results were obtained. Even it is observed that, foods with ostrich eggs were preferred more.

As a result, it is thought that, ostrich egg can be used in pastry making and catering sectors since it is similar to chicken egg. However, its price is 100 time that of a chicken egg, as it is a recent sector in our country and egg production is low. This case contributes negatively of the use of ostrich egg in food sector.

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INFLUENCE OF HENS' DIET ON YOLK CHOLESTEROL, FATTY ACID PROFILE, CAROTENOID PIGMENTS, VITAMIN E AND SELENIUM LEVELS

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Keywords : egg cholesterol, carotenoid pigments, fatty acids, selenium, spirulina

Abstract

WLH hens were fed with a control and three functional diets; namely FSE=150g flaxseeds + 200mg vitamin E + 3g Spirulina/ kg, FOSe=20g fish oil + 0.2mg organic selenium (Sel-plex) + 3g Spirulina/ kg and FSE+FOSe=75g flaxseed + 10g fish oil + 100mg vitamin E + 0.1mg organic selenium + 3g Spirulina/ kg diet from 53 to 58 weeks of age. Supplementation of Spirulina had significantly increased the yolk carotenoid pigment levels, compared to the control. The three functional diets had reduced the yolk cholesterol levels by 22-26 percent, compared to control. Boiled eggs from all the four treatments had comparable organolepic acceptability. All the three functional feeds had significantly increased the n-3 fatty acid levels in the egg yolk. Dietary selenium and vitamin E levels had acted synergistically in increasing their levels in the eggs.

Resumé

Les poules de WLH ont été nourries avec un aliment témoin et trois régimes fonctionnels; à savoir FSE=150g de graines de lin + 200mg de vitamine E + 3g de spiruline/kg ; FOSe=20g d'huile de poisson + 0.2mg de sélénium organique (Sel plex) + 3g de spiruline/kg ; et FSE+FOSe=75g de graines de lin + 10g d'huile de poisson + 100mg de vitamine E + 0.1mg de selenium organique + 3g de spiruline/kg, de 53 à 58 semaines. La supplémentation en spiruline a augmenté significativement les niveaux de pigment caroténoïdes du jaune, en comparaison du témoin. Les trois régimes fonctionnels ont réduit les niveaux de cholestérol de jaune de 22-26 %. Les œufs bouillis issus des quatre alimentations de tous les quatre traitements ont des qualités organoleptiques comparables. Les trois alimentations fonctionnelles nourrit

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significativement ont augmenté significativement les niveaux en acides gras n-3 dans le jaune d'œuf. Les alimentations enrichies en selenium et en vitamine E ont agi synergétiquement pour augmenter leurs niveaux dans les oeufs.

Introduction

Eventhough the egg is the most nutritious food and relatively cheaper, the egg consumption in many developing countries is lower due to cholesterol scare. Hence many scientists have started working to reduce the egg and serum cholesterol levels. Basmacioglu *et al.* (2001) had successfully reduced the yolk cholesterol levels by flaxseed feeding. Anderson *et al.* (1991) improved the yolk colour by feeding spirulina. Cherian and Sim(1993), Galobart *et al.* (2001) and Narahari *et al.* (2003) enriched the eggs with omega-3 fatty acids and antioxidants several fold. Here an attempt has been may to incorporate various health promoting components in the egg and to reduce the yolk cholesterol by feeding different functional feeds.

Material and methods

Ninety six, Forsgate strain WLH hens in cages were randomly divided into 16 groups of six hens each. Four replicates were randomly alloted to each of the four treatments. They were fed *ad libitum* with a control and three functional feeds, namely **FSE=**Layer mash containing 150g ground full fat flaxseed + 200mg supplemental vitamin E + spirulina 3g/ kg diet, **FOSe=**Layer mash containing 20g Anchovy fish oil + organic selenium (Sel-plex) to supply 0.2mg selenium + spirulina 3g/ kg diet and **FSE + FOSe=**Layer mash containing 75g ground full fat flaxseed + 10g Anchovy fish oil + 100 mg vitamin E + 0.1 mg organic selenium + spirulina 3g/ kg diet from 53 to 58 weeks of age. The ingredient and chemical compositions of the four experimental feeds are shown in Tables 1and 2, respectively.

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Table 1. Ingredient composition of the experimental layer feeds (g / kg)

Ingredient	*Control*	*Flax Seed + Vit. E (F.S.E.)*	*Fish oil + Org.Se. (F.O.Se)*	*FSE + FO Se*
Corn	300	180	277	200
Broken rice	200	205	200	230
Rice polish	100	--	30	12
Deoiled rice bran	--	100	60	85
Soybean meal	200	122	200	175
Sunflower meal	100	140	110	110
Dicalcium phosphate	10	10	10	10
Shell grit	88	87.6	87.9	87.75
Flax seed	--	150	---	75
Fish oil	--	---	20	10
Trace mineral premix	1	1	1	1
Vitamin premix	1	1	1	1
Ethoxyquin	+	+	+	+
50% Vitamin -E	--	0.4	--	0.2
Organic selenium	--	--	0.1	0.05
Spirulina	-	3	3	3

Table 2. Nutrient composition of the experimental diets

Nutrient	*Control*	*F.S.E.*	*F.O.Se*	*FSE + FOSe*
CP (g/ kg)	172.0	173.5	172.3	173.2
ME (MJ/ kg)	11.05	11.08	11.07	11.09
Lipids (g/ kg)	37.5	69.1	54.7	63.5
Calcium (g/ kg)	34.5	34.2	33.9	33.8
Total phosphorus (g/ kg)	7.1	7.4	7.2	7.6
Lysine (g/ kg)	7.6	7.4	7.6	7.5
Methionine (g/ kg)	3.1	3.1	3.1	3.0
Vitamin E (mg/ kg)	29.2	219.8	96.6	160.2
Selenium (mg/ kg)	0.090	0.085	0.293	0.196
Total carotenoid pigments (mcg/ g)	6.5	18.2	19.1	18.8

During the last week of the study, two eggs from each replicate were collected and the yolk samples were assayed for their cholesterol (Washburn and Nix, 1974), carotenoid pigments (AOAC, 1990), Fatty acid composition (Wang *et al.,* 2000) and vitamin E (Abdollahi *et al.,* 1993). Two samples of yolk and albumen from each replicate were

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analysed for their selenium content, using an atomic absorption spectrophotometer, aspect the method of Canter and Tarino (1982).
The organoleptic acceptability of the hard boiled eggs from the four treatments, were assessed by 12 taste panelists for their colour, texture, taste, flavour and over all acceptability, using a four point hedonic scale.
All the data collected were subjected to analysis of variance for a completely randomized design according to the methods of Snedecor and Cochran (1989). The significance was tested using Duncan's multiple range test (Duncan, 1955).

Results and Discussion

The dietary effects on various treatments are shown in Tables 3 and 4. All the three functional feeds had significantly ($P<0.01$) reduced the yolk cholesterol levels and increased the yolk carotenoid pigments, colour, vitamin E and selenium levels. The yolk cholesterol reduction in this study was more than 25 per cent; which is highest than the values reported by Scheideler and Froning (1996) and Basmacioglu *et al.* (2001). This was probably due to the combined effects of omega-3 fatty acids, vitamin E, organic selenium and spirulina.

Spirulina supplementation in the functional feeds had significantly ($P<0.01$) contributed to the rich orange yolk colour; due to high carotenoid pigment levels. Ross and Dominy (1990) and Anderson *et al.* (1991) also used spirulina in the hens' diet to increase the yolk colour.

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Table 3. Effect of functional feeds on yolk cholesterol, carotenoid pigments, vitamin E, egg selenium and organoleptic[1] traits

Trait[@]	*Control*	*F.S.E.*	*F.O.Se*	*FSE + FO Se*
Yolk cholesterol (mg/g)**	12.9^{b}±0.03	9.9^{a}±0.09	9.5^{a}±0.12	10.0^{a}±0.19
Yolk carotenoid pigments (µg/g)**	24.5^{b}±0.12	65.1^{a}±0.03	65.8^{a}±0.07	65.0^{a}±0.04
Vitamin-E** (µg/g yolk)	96.8^{d} ± 3.77	296.5^{a} ± 7.98	169.8^{c} ± 6.09	237.5^{b} ± 4.66
Selenium **in egg white (ng/g)	51.25^{c} ± 1.70	45.50^{c} ± 3.50	99.25^{a} ± 3.20	85.75^{b} ± 1.65
Selenium** in yolk (ng/g)	188.0^{c} ± 8.29	177.5^{c} ± 3.75	420.3^{a} ± 6.28	356.0^{b} ± 5.16
Flavour^{NS}	2.3 ± 0.42	3.2 ± 0.17	2.5 ± 0.34	2.6 ± 0.33
Colour**	1.8^{b} ± 0.48	3.0^{a} ± 0.33	3.0^{a} ± 0.36	3.3^{a} ± 0.29
Taste and texture^{NS}	3.0 ± 0.42	3.5 ± 0.29	3.5 ± 0.28	3.7 ± 0.27
Overall acceptability^{NS}	3.0 ± 0.23	3.2 ± 0.21	3.0 ± 0.23	2.9 ± 0.13

@= Means within each row bearing different superscripts differ significantly;
1=Organoleptic score 1=least acceptable and 4= highly acceptable.

Table 4. Effect of functional feeds on fatty acid composition of yolk lipids (g/ 100g of total fatty acids)

Fatty acid[@]	*Control*	*F.S.E.*	*F.O.Se*	*FSE + FO Se*
$C_{14:0}$**	0.38^{c} ± 0.03	0.25^{b} ± 0.04	0.19^{a} ± 0.02	0.19^{a} ± 0.03
$C_{16:0}$**	27.48^{c} ± 0.11	19.91^{a} ± 0.66	24.23^{a} ± 0.66	20.08^{a} ± 0.10
$C_{16:1}$**	3.60^{c} ± 0.07	2.4^{bc} ± 0.13	2.25^{b} ± 0.08	1.87^{a} ± 0.04
$C_{18:0}$**	7.57^{a} ± 0.05	7.73^{b} ± 0.36	8.13^{ab} ± 0.06	7.90^{ab} ± 0.05
$C_{18:1}^{NS}$	44.43^{a} ± 0.10	43.74^{a} ± 1.18	44.61^{a} ± 0.50	43.24^{a} ± 0.17
$C_{18:2}$**	15.67^{c} ± 0.05	14.58^{b} ± 0.10	15.35^{c} ± 0.14	14.07^{a} ± 0.05
$C_{18:3}$**	0.16^{c} ± 0.02	7.57^{a} ± 0.24	0.39^{c} ± 0.02	6.04^{b} ± 0.10
$C_{20:5n-3}$**	0.06^{d} ± 0.01	0.35^{c} ± 0.02	0.61^{a} ± 0.04	0.48^{b} ± 0.02
$C_{22:6n-3}$**	0.17^{d} ± 0.01	2.70^{c} ± 0.17	6.81^{a} ± 0.20	3.69^{b} ± 0.14

@= Means within each row bearing different superscripts differ significantly

The vitamin E level in yolk is not only directly proportional to the dietary vitamin E level; but also to the dietary organic selenium (Sel-plex) level. Jiang *et al.* (1994) Cherian *et al.* (1996), Grobas *et al.* (2002) and Narahari *et al.* (2003) also observed

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linear relationship between dietary and yolk vitamin E levels. Significant increase in yolk vitamin E levels in FOSe treatments, where vitamin E was not supplemented through feed; but only organic selenium was added, stongly suggested the synergistic and sparing action of selenium, to accumulate more vitamin E in yolk. As observed earlier by Cantor *et al.* (2000) and Surai and Dvorska (2000), significant increase in the egg selenium level was due to the dietary organic selenium supplementation. Yolk has accumulated nearly four times more selenium than albumen.

Except the colour, due to the pigments, other organoleptic traits were comparable between the treatments; suggesting that the eggs from both control and functional feeds fed hens had equal consumer acceptability.

The omega-3 fatty acid levels in the yolks from hens fed functional feeds had increased by 18 to 21 fold, compared to the control; which concur with the earlier findings of Balevi and Coskun (2001), Galobart *et al.* (2001) and Narahari *et al.* (2003). As suggested by Narahari *et al.* (2003), the spirulina might have acted synergistically in increasing few O-3 fatty acid levels in yolks.

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IMPROVING THE NUTRITIVE VALUE OF EGGS BY FEEDING LAYING HENS AN OPTIMUM VITAMIN NUTRITION (OVN™)

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Keywords: optimum, vitamin, nutrition, egg, quality

Abstract

Eggs are an excellent source of high quality and easily digestible protein as well as a good source of certain vitamins and minerals. Levels of vitamins in eggs are directly linked to the levels in the diet of the hen. Layer feed conversion rates have improved drastically (approx. 40%) during last 30 years so that it is essential to re-evaluate accordingly vitamin levels in feed if we want to provide eggs to consumers with, at least, the same nutritional value than in the past. This paper summarises the effects of 2 vitamin levels in laying hen diets (average level used in Spain vs. Optimum Vitamin Nutrition level) on the vitamin deposition and quality of eggs. Eggs from hens fed OVN™ diets had significantly higher concentration of vitamins A, E, B1, B2, B12, folic acid, pantothenic acid and biotin, resulting in eggs with a higher nutritional value than their control counterparts.

Résumé

Les œufs sont une excellente source de protéines facilement digestibles et constituent aussi une source importante de plusieurs vitamines et minéraux. Les niveaux de vitamines dans les œufs sont liés aux teneurs en vitamines de l'aliment des pondeuses. Au cours des 30 dernières années, l'efficacité alimentaire des pondeuses s'est beaucoup améliorée (d'environ 40%), il devient donc essentiel de re-visiter la supplémentation vitaminique de l'aliment afin de proposer aux consommateurs des œufs qui présentent la même valeur nutritionnelle que par le passé. La présente publication montre les effets de deux niveaux vitaminiques chez les pondeuses sur la déposition des vitamines et la qualité des œufs. Le niveau vitaminique témoin, reflet de la supplémentation moyenne réalisée en Espagne, est comparé au niveau OVN™

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recommandé par Roche. Le niveau OVN™ a conduit à une augmentation significative des teneurs de vitamines A, E, B1, B2, B12, acide folique, acide pantothénique et biotin dans les œufs par rapport au groupe témoin, résultant en des œufs de valeur nutritionnelle supérieure à celle des œufs des pondeuses témoin.

Introduction

Eggs are one of the most complete and versatile foods currently available, being an excellent source of high quality and easily digestible protein as well as a good source of certain vitamins and minerals. Poultry vitamin levels in feed have been historically established by NRC (1994) and ARC (1984) to prevent clinical deficiencies and have changed only little over the last 40 years. During the same period of time, layer feed conversion rates have improved drastically (approx. 40%) based on a higher egg mass production (approx. 30%) and lower feed consumption (approx. 10%), what makes necessary adapt accordingly vitamin levels in feed.

An optimum level of vitamins in feed is essential to allow birds to achieve their full genetic potential and enhance health, welfare and therefore productivity. But also the egg industry should not forget that levels of vitamins in eggs are directly linked to the levels in the diet of the hen, contributing to make the egg such a nutritive food product.

Nowadays, safe, tasty, nutritious and appetising food products are demanded by consumers. Retailers attach considerable importance to satisfying these demands. This involves continuous product development to create new value added products. Supporting these assumptions, recent consumer surveys in Spain (Hernandez et Seehawer, 2001) have shown that consumers consider the nutritive value of eggs as one of the most important egg quality factors.

All these facts motivated some research where the effect of using 2 different vitamin levels in layer feed was evaluated. On the one hand, the average vitamin levels used by Spanish egg producers and on the other hand, levels recommended by several worldwide specialists (Barroeta et al, 2002) and described in this paper as Optimum Vitamin Nutrition levels (OVN™).

Material and Methods

The purpose of the trial was to study the effect of using different vitamin levels in laying hen diets on vitamin deposition and on egg quality. Layers were subjected or

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not to environmental stress conditions as produced by a restricted ventilation rate which increased air ammonia concentration.

One hundred and twenty eight brown hens of the Hy-Line strain were used. There were four experimental treatments replicated 16 times and allocated at random by blocks, according to the location in the experimental rooms. The criterion used in choosing blocks was rate of lay, evaluated in the pre-experimental period. Hens of treatments T-1 and T-2 were reared in a room under normal conditions, while layers of treatments T-3 and T-4 were subjected to environmental stress conditions, produced by a restriction on ventilation rate, that produced an increase in air ammonia concentration. Layers from treatments T-1 and T-3 were fed a vitamin premix (Table 1) formulated according to the usual practice in Spain (Villamide et Fraga, 1999), whereas birds from treatments T-2 and T-4 were fed higher vitamin levels described in this paper as Optimum Vitamin Nutrition levels (OVN™).

The duration of trial included a pre-laying period followed by four periods of four weeks each of laying period (from week 22 to week 38). Egg production, egg weight, mortality, feed consumption, feed efficiency and egg quality parameters were evaluated.

Four different pools of eggs (yolk + white) for each treatment were prepared and sent to Roche Vitamins Ltd. laboratories (Basel) to analyse their vitamin content. The oxidative stability of egg yolk was assessed by measuring the non-induced TBARS (Thiobarbituric Acid Reactive Substances) values of 16 egg samples per treatment

Table 1. Composition of experimental vitamin premixes

Vitamins	T1, T3: Control (mg/kg)	T2, T4: OVN (mg/kg)
A: (Retinol)	8110 UI (16.22 g)	12000 UI (24 g)
D3: Cholecalciferol	2160 UI (4.32 g)	3500 UI (7 g)
E: alfa-Tocopherol	6.90	50
K3: Menadione	1.43	3.00
B1: Thiamin	0.91	3.00
B2: Rivoflavin	3.94	7.00
B6: Piridoxal	1.63	5.00
B12: Cobalamine	0.0123	0.025
PP (B3): Niacin	21.40	50.00
B5: Pantothenic acid	7.45	10.00
M: Folic acid	0.31	1.00
H: Biotin	0.02	0.15
C: Ascorbic acid	0	100

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Results and discussion

Data of vitamin contents found in egg samples are reported in Table 2. As it can be observed, almost all vitamin levels (A, E, B1, B2, B12, pantothenic acid, folic acid, biotin) determined in whole egg were statistically increased when hens were fed OVN premix (but not enough to be considered as vitamin enriched eggs), thus improving in that way the nutritive value compared to control eggs. Vitamin C was not deposited in eggs in any of the 4 treatments which is in line with previous unpublished research.
It is interesting to observe that standard eggs produced in Spain (eggs from T-1 and T-3) show a lower level of some vitamins (Figures 1, 2, 3) compared to those levels published for eggs in the official nutritional tables (MAPA, Spanish Ministry of Agriculture, 1995). This fact is probably related to the improvement of layer feed conversion due mainly to better animal genetics and management compared to the past and the non-corresponding adaptation of vitamin levels in feed: the lower the vitamin intake by the hen, the lower the vitamin deposition in the egg. On the other hand, OVN eggs contained a higher level of those vitamins which allowed eggs have a nutritional value close to the one published in official nutritional tables.

Table 2. Vitamin content determined in whole eggs

Treatment	Vitamin premix	Room	Stress	Vit. A (IU/kg)	Vit. E (ppm)	Vit. B1 (ppm)	Vit. B2 (ppm)	Vit.. B12 (ppm)
T-1	Control	1	No	5905	13.38	0.52	4.05	14.38
T-2	OVN	1	No	6147	40.00	0.58	4.08	20.30
T-3	Control	2	Yes	5660	12.50	0.52	3.85	14.08
T-4	OVN	2	Yes	6222	46.12	0.72	4.15	24.35
Premix effect:			Control	5782 b	12.94 b	0.52 b	3.95 b	14.22 b
			OVN	6185 a	43.06 a	0.65 a	4.11 a	22.32 a
			Pr>F	***0.0013***	***0.0001***	***0.0022***	***0.0263***	***0.0001***
			St.Err.	*68*	*0.81*	*0.02*	*0.04*	*0.42*

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Treatment	Vitamin premix	Room	Stress	Ca-panto-thenate (mg/kg)	Folic acid (mg/kg)	Biotin (micro g/kg)	Vit. C (mg/kg)
T-1	Control	1	No	15.60	0.51	229.50	0
T-2	OVN	1	No	18.38	0.71	323.50	0
T-3	Control	2	Yes	14.30	0.47	210.00	0
T-4	OVN	2	Yes	18.62	0.64	326.25	0
Premix effect:		Control		14.95 b	0.49 b	219.8 b	0
		OVN		18.50 a	0.67 a	324.9 a	0
		Pr>F		***0.0029***	***0.0001***	***0.0001***	-
		St.Err.		*0.67*	*0.02*	*4.79*	-

In Table 3, TBARS values determined in egg yolk collected right at the end of the experiment are presented. As it can be seen, lipid oxidation of egg yolks was not statistically affected by type of vitamin-premix, although eggs of hens fed OVN premix showed numerically lower TBARS values (0.274 *vs* 0.301 nmol/g) than animals fed control- diets. These results agree with the level of antioxidant vitamin E found in OVN feeds and also with the level of vitamin E in whole eggs (43.06 ppm in eggs of layers fed OVN premix versus 12.94 ppm found in eggs of hens fed control premix). In general, no significant differences on laying hen performance were obtained between birds fed different levels of vitamins.

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Table 3. TBARS values determined in egg yolk at the end of the experiment

Treatment	Vitamin premix	Room	Stress	TBARS values (nmol/g)
T-1	Control	1	No	0.308
T-2	OVN	1	No	0.262
T-3	Control	2	Yes	0.294
T-4	OVN	2	Yes	0.285
Premix effect:		Control		0.301
			OVN	0.274
			Pr>F	*0.3088*
			St.Err.	*0.018*

Figure 1. Vitamin A content in whole egg (mg retinol/100 g)

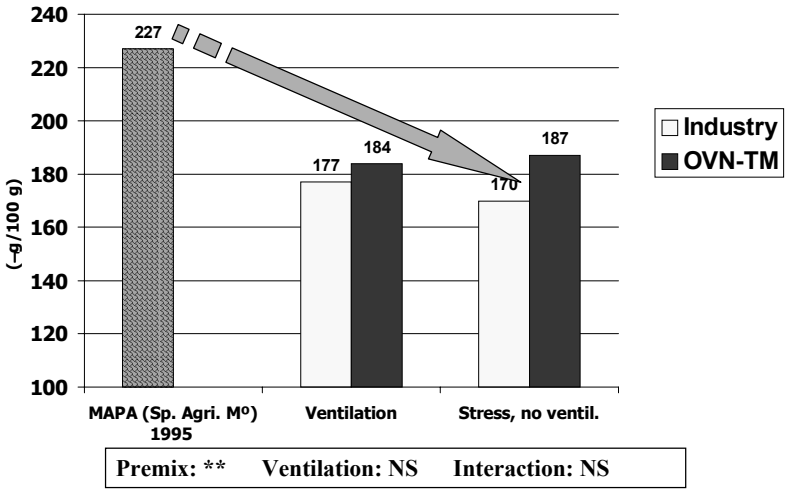

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Figure 2. Vitamin E content in whole egg (mg/100 g)

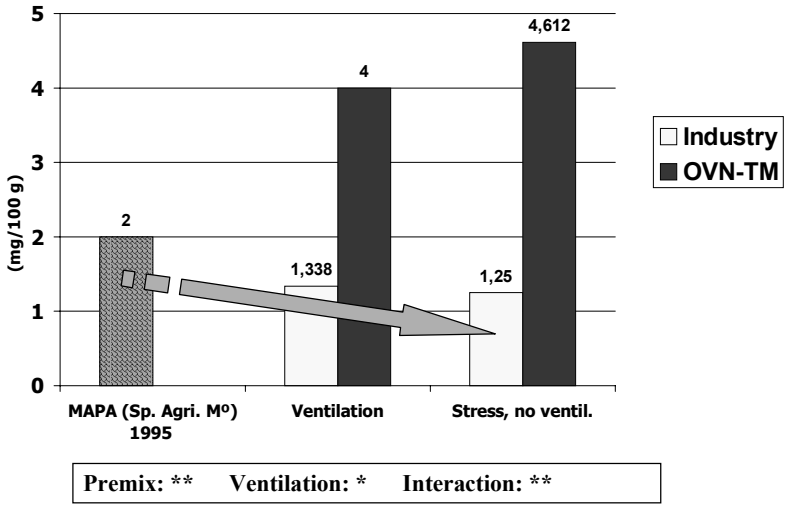
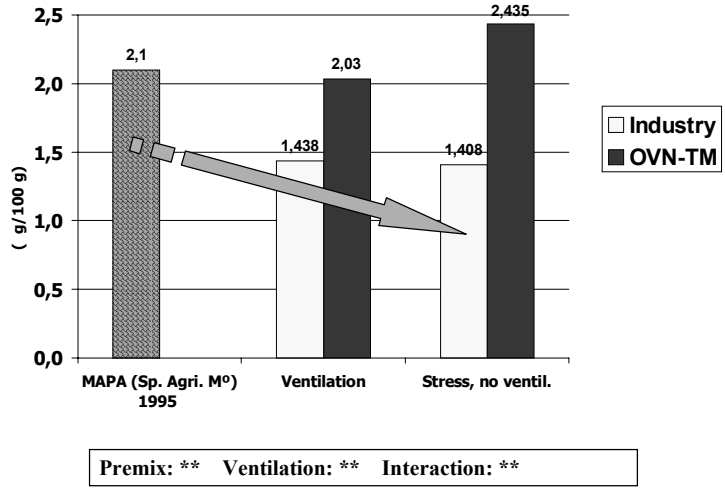


Figure 3. Vitamin B_{12} content in whole egg (mg/100 g)



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THE EFFECTS OF OMEGA-3 FATTY ACIDS, IODINE AND SELENIUM SUPPLEMENTATION OF LAYING HEN FEED ON THE EGG QUALITY

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Keywords : Omega-3 fatty acid, iodine, selenium, egg quality, laying hen.

Abstract

The authors examined the effects of omega-3 fatty acids, with and without a supplement of iodine and selenium, on the zootechnical performances of laying hens and on egg quality. One hundred and twenty six birds were allocated to seven groups of 18 hens each. For 6 weeks animals received a complete diet: C) control; L) 1% linseed oil; A), AJ), ASe) and AJSe) 1.67% micro-algae (Omega Tech Gmbh) (A) and 5.5 ppm potassium iodine (J), 2.03 ppm sodium selenite (Se) or both salts; F) 4.95% fish oil. A higher ($P< 0.05$) feed consumption (g/d) was observed in groups A and AJ than in groups L, AJSe and F (128.2 and 121.1 *versus* 114.4, 112.9, and 110.4, respectively). Cholesterol was not influenced by lipidic sources. DHA was higher ($P< 0.01$) in groups AJSe and L.

Résumé

Ont été étudiés les effets des acides gras omega-3, additionnés ou non d'iode et de sélénium, sur les performances zootechniques de poules pondeuses et sur la qualité des œufs. 126 animaux ont été subdivisés en 7 groupes de 18 sujets chacun. Pendant 6 semaines, les poules ont reçu une alimentation complète sans ajouts (C) ou additionnée de: L) 1% d'huile de lin; A), AJ), Ase) et AJSe) 1,67% de micro-algues (Omega Tech Gmbh) (A) et 5,5 ppm de iodure de potassium (J), 2,03 ppm de sélénite de sodium (Se) ou des deux sels; F) 4,95% d'huile de poisson. Dans les groupes A et AJ, on a pu observer une consommation plus élevée ($P< 0,05$) (g/j) que dans les groupes L, AJSe et F (128,2 et 121,1 contre 114,4, 112,9, et 110,4). Le cholestérol n'a subi l'influence d'aucune source lipidique. Le DHA était plus élevé ($P< 0,01$) dans les groupes AJSe et L.

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Introduction

It is recognised that most so-called lifestyle disease associated with high risk arise from common dietary errors, especially in Western societies. This is evident in the excessive consumption of fat and in its unfavourable composition in the diet. Over the last twenty years many findings have shown that a correct proportion among fatty acids (FA) - i.e. saturated, monounsaturated and polyunsaturated (PUFA) of n-3 and n-6 - provides beneficial health effects. N-3 FA are essential for normal growth and important for brain development as well as for the prevention and treatment of cardiovascular disease and certain cancers (Durgham and Fernandes, 1996; Lauritzen et al., 2001; Williams, 2000). Simopoulos (2001) suggested that an intake of 650 mg per day is desirable for adults. Unfortunately, the intake of n-3 PUFA is usually low and does not reach the recommended levels, which may be esteemed as 0.8-1.4 g for eicosapentaenoic acid (EPA) and docosahexaenic acid (DHA), or 3-5.5 g for total n-3 PUFA per day (British Nutrition Foundation, 1992). One way to increase the amount of n-3 long chain PUFA in the diet is by consuming fish (200-300g fish per week), fish oil or food enriched with EPA and DHA, generally referred to as "functional food". Increasing the DHA content of animal products is also important because it is retained in the human body to a greater degree than EPA; DHA levels in plasma phospholipids remain elevated for up to 14 weeks after intake of DHA is stopped (Hodge et al., 1993; Marangoni et al., 1993). Algae are the original source of n-3 PUFA in the diet of fish. Recent developments in n-3 PUFA research include a technology for producing natural marine micro-algae with a high DHA content via fermentation. Dried micro-algae provide naturally encapsulated n-3 FA for feed supplementation. Oxidative deterioration of lipids, particularly PUFA promotes adverse flavour changes in foods and consumption of foods/lipids that have undergone oxidation may also contribute to the development of chronic disease. Iodide plays two important roles: antioxidant and hormonal. This trace element is strictly connected to two PUFA (arachidonic acid and DHA) to form specific iodolipids. As Cocchi and Venturi (2000) observed, cooperation between iodine and selenium exists and selenium is present in peroxidase enzymes, which are able to oxidise iodides. Selenium can be concentrated in eggs by transfer from the diet; thus eggs can provide enriched sources. This study aimed to establish whether it was feasible to enrich eggs with n-3 PUFA using a DHA-rich algal product instead of fish or linseed oil, with or without a supplement of iodide and/or selenium. The effects of these diets on both zootechnical and egg quality and performances were examined.

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Material and Methods

One hundred and twenty six 18-week-old Warren laying hens were divided into seven experimental groups of 18 birds each (6 replicates of 3 hens each). Throughout six weeks, animals received a complete isonitrogenous and isoenergetic: C) control: complete diet; L) diet containing 1% linseed oil; A); AJ); ASe) and AJSe) diets containing 1.67% DHA-rich oil from the micro-algae *Schizochytrium* sp. (Omega Tech Gmbh) (A) and 5.5 ppm potassium iodine (J), 2.03 ppm sodium selenite (Se) or both the salts (JSe); F) diet containing 4.95% fish oil. Feed and water were provided *ad libitum* and feed consumption was determined each week. Egg qualitative and chemical parameters were determined in eggs laid during a three-day period at 0 and at 42 days from the start of the treatment: egg weight, percentage of shell, albumen and yolk, Haugh index (HU), yolk colour, measured using a Minolta Chomometer Reflectance CR 200/08 (Barletta Apparecchi Scientifici S.r.l., Milan, Italy) with D65 Illuminant, and shell thickness. Colour number (CN) was determined according the method proposed by Scholtyssek (1995). The chemical composition of the yolk was determined using AOAC (1995) methods: 950.46, 984.13, 925.32, 942.05 to assess moisture, N x 6.25, ether extract and ash respectively. Cholesterol and FA were extracted (Adams et al., 1986; Bligh and Dyer, 1959) and analysed by gas chromatography with a SPB-1 and SP-2380 fused silica capillary column respectively. Data were subjected to analysis of variance and means compared by the *t* Student's test.

Table 1. Fatty acid composition of diets (%, w/w)

	Treatment						
Fatty acid	C	L	A	AJ	ASe	AJSe	F
C14:0	0.30	0.19	1.69	1.57	1.44	1.90	3.36
C16:0	14.22	12.94	15.86	17.15	16.61	17.01	15.85
C16:1n-7	0.30	0.14	0.17	0.62	0.18	0.50	2.39
C18:0	2.92	3.06	2.82	3.39	3.00	2.84	2.54
C18:1n-9	23.96	21.88	18.76	19.25	20.14	19.78	16.70
C18:2n-6	54.33	49.44	47.79	47.85	49.04	47.14	39.48
C18:3n-3	2.04	11.19	4.89	2.61	2.43	2.25	3.92
C20:0	0.27	0.43	0.27	0.25	0.24	0.26	0.14
C20:5n-3	0.27	0.01	0.86	0.23	0.82	0.80	4.56
C22:6n-6	0.01	0.00	1.20	1.40	1.02	1.23	0.04
C22:6n-3	0.29	0.01	4.82	4.05	4.23	5.06	5.67

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Table 2. Zootechnical performances

	Treatment							
	C	L	A	AJ	ASe	AJSe	F	SEM
FI* g/d	119.1ab	114.4a	128.2b	121.1b	121.1ab	112.9a	110.4a	1.6
n-3*	160	700	760	500	580	500	1080	
EP* %	46.3	39.7	45.7	46.6	41.2	49.7	43.6	1.2

*FI: Feed intake; n-3 ingested (mg/head/d); EP: Egg production; a, b: $P < 0.05$

Results and discussion

No significant differences in hen weight or weight change were detected among treatments at the end of the trial. During the experimental trial, egg production showed an elevated variability and thus appeared not to be influenced by diets. Feed intake showed some differences: hens of group L, AJSe and F swallowed less feed than hens in groups A and AJ (table 2). It was supposed that these results might be correlated with the unpleasant taste of feeds. At the beginning of the trial egg quality parameters did not differ between groups, hence the data are not shown. Egg weight and shell, albumen and yolk percentages were influenced by treatments (table 3): smaller eggs with a higher percentage of shell and a reduction in albumen ($P< 0.01$) were observed in groups C, AJSe and Herbert and Van Elswyk (1996) observed a transient depression of egg weight in 24-week-old hens fed a diet supplemented with 1.5% menhaden oil or 4.8% micro-algae; this change in whole egg and yolk weights was not present in 52-week-old hens, so it appeared that the reproductive maturity of the birds decreased their vulnerability to the effects of dietary n-3 FA on egg weight. Groups AJSe and F also showed a greater shell thickness ($P< 0.05$), whereas HU and CN were not influenced by the diets. The pH value was always at the same levels in every treatment, even though a lower value ($P <0.05$) appeared in egg albumen of hens fed on diet supplemented with micro algae and iodide. Chemical analyses of yolk showed the same values in all the groups; only a high value of crude protein appeared in group AJ (table 3). Cholesterol was not influenced by the different lipidic sources, even at the highest level of incorporation of group F. The total cholesterol content was consistent with the data reported by other authors, when expressed in mg/g yolk (Meluzzi et al., 1998). As would be expected, the polyunsaturated profile of the yolk was significantly influenced by diet. Yolks from hens on diets L, A, AJ, AJSe and F exhibited significant reductions in n-6 FA content in response to dietary n-3 FA (C18: 3 n-3 *plus* C 20:5 n-3 and C22: 6n-3) (tables 1, 2 and 5). DHA was higher in groups AJSe and F. The observed alterations in the FA profile is not surprising, as previous studies have shown that increasing the dietary n-3 FA level in poultry feeds results in a decreased yolk n-6 FA content (Herbert and Van Elswyk, 1996; Meluzzi et al., 2000).

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Leskanich and Noble (1996) reviewed the research in this field which focused specifically on the FA enrichment in poultry eggs and meat. The drawback of this enrichment was the unacceptable taste and odours that developed in eggs and meat from birds fed diets containing fish oil. Use of a microalgal- based DHA feed supplement for poultry can solve this problem The DHA- rich oil in the cells is naturally encapsulated by the cell walls. The presence of canthaxantin and β-carotene in the cells probably contributes to the stability of the DHA- rich oil in the cell. Antioxidants such as iodide and selenium can be added to enhance product stability and shelf life of PUFA. Because of the very high concentration of DHA in *Schizochytrium* sp. cells, only a small amount of the micro-algae needs to be added to feed in order to increase the DHA content of eggs; no other changes in eggs will result.

Table 3. Egg quality parameters at the end of the trial

	Treatment							
	C	L	A	AJ	ASe	AJSe	F	SEM
EW *	57.3A	65.2B	66.7B	66.3B	65.4B	60.0A	57.6A	0.4
Y*	23.9B	23.8B	23.1AB	22.4A	23.8B	23.5B	23.3B	0.1
S*	11.9BC	10.9A	11.2A	10.9A	10.9A	11.7AB	12.1C	0.05
Alb*	64.1A	65.3B	65.7B	67.1C	65.2B	64.8A	64.6A	0.1
HU*	102.3	103.6	101.8	102.0	101.4	101.8	103.1	0.5
ST*	0.357b	0.336a	0.362b	0.352b	0.353b	0.365c	0.367c	0.002
CN*	9.8	9.5	9.8	9.8	9.6	9.7	9.9	0.07
pH	8.5b	8.4b	8.4b	8.2a	8.4b	8.3ab	8.4b	0.07

*EW: egg weight (g); Y: yolk (%); S: shell (%); Alb.: albumen (%); HU: Haugh units (n); ST: shell thickness (mm); CN: colour number (n); A, B, C: $P< 0.01$; a, b, c: $P < 0.05$.

Table 4. Chemical composition of the yolk at the end of the trial (% DM*)

	Treatment							
	C	L	A	AJ	ASe	AJSe	F	SEM
DM*	52.17	52.16	52.02	48.67	53.18	51.57	49.91	0.22
CP*	30.44a	31.54a	32.73ab	33.25b	31.00a	32.44a	31.50a	0.22
EE*	65.02	62.80	62.24	61.81	62.30	60.58	62.31	3.84
Ash	2.85	3.02	2.96	3.04	2.96	2.93	2.95	0.15
Chol.*	177.42	195.74	187.36	163.26	219.27	193.73	202.99	6.47

*DM: dry matter; CP: crude protein; EE: ether extract; Cholesterol (mg/egg); a, b: $P < 0.05$.

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Table 5. Fatty acid composition of the yolk at the end of the trial (%, w/w)

	Treatment							
	C	L	A	AJ	ASe	AJSe	F	SEM
C14:0	0.37A	0.40A	0.46B	0.50BC	0.46B	0.55CD	0.73D	0.02
C16:0	27.04A	26.82A	27.93A	28.58B	27.51A	28.95B	28.78B	0.17
C16:1n-7	2.09A	2.68B	2.99B	3.05B	2.29A	2.52A	2.96B	0.07
C18:0	9.27B	9.15B	8.20A	8.01A	8.56AB	9.14B	9.39B	0.13
C18:1n-9	37.03A	40.25B	39.82B	39.63B	38.03AB	37.26A	37.03A	0.33
C18:2n-6	19.93B	15.61A	15.19A	15.01A	18.29B	15.58A	14.77A	0.37
C18:3n-3	0.41A	1.39E	0.74C	0.59B	0.48A	0.39A	0.82D	< 0.01
C20:0	0.14C	0.104B	0.08A	0.08A	0.09AB	0.08A	0.07A	<0.01
C20:1	0.17A	0.17A	0.18A	0.18A	0.19AB	0.22B	0.41C	0.05
C20:4n-6	1.86C	1.56B	1.22B	1.16B	1.25B	0.97A	0.49A	0.01
C22:4n-6	0.26C	0.12B	0.10B	0.10B	0.11B	0.10B	0.05A	0.07
C22:5n-3	0.13A	0.15B	0.13A	0.12A	0.09A	0.31C	0.53C	0.01
C22:5n-6	0.60E	0.16B	0.26D	0.26D	0.23CD	0.22C	0.04A	0.03
C22:6n-3	0.69A	1.44B	2.69C	2.73C	2.43C	3.73D	3.92D	0.19

A, B, C, D, E: $P < 0.01$

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EGG SELENIUM CONCENTRATIONS IN BREEDER HENS FED DIETS WITH SELENIUM DERIVED FROM DIFFERENT SOURCES

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Keywords : Sel-Plex, Selenium , Enriched, Eggs, Breeder

Abstract

The aim of these studies was to determine the effect of selenium from Sel-Plex® (Alltech Inc.) on the transfer of selenium into the egg of broiler breeder hens under commercial conditions. Two randomized block design trials were conducted on 9 and 6 farms (2 houses per farm), repectively. In trial 1 diets were supplemented with Na-selenite (0.2ppm Se) or Sel-Plex® (0.2ppm Se). In trial 2 diets were supplemented with Na-selenite (0.2ppm Se) or a combination of Na-selenite (0.2ppm Se) and Sel-Plex® (0.2ppm Se). In both trials the treatment containing Sel-Plex® led to a significant increase ($P<0.05$) in selenium concentrations in the yolk, albumen and total egg compared to the control.

Résumé

Le but de cette étude était de déterminer l'effet de la forme de sélénium provenant du Sel-Plex® (Alltech Inc.) sur le transfert du sélénium dans l'œuf de poules reproductrices conduites dans des conditions d'élevage commercial. Deux essais en blocs aléatoires furent réalisés dans respectivement 9 et 6 élevages (2 bâtiments par élevage). Dans l'essai N°1 les rations furent supplémentées en sélénite de soude (0.2 ppm de Se) ou en Sel-Plex® (0.2 ppm de Se). Dans l'essai N°2 les rations furent supplémentées en sélénite de soude (0.2 ppm de Se) ou avec une combinaison de sélénite de soude (0.2 ppm de Se) et de Sel-Plex® (0.2 ppm de Se). Dans les deux essais le traitement contenant du Sel-Plex® a montré une augmentation significative ($P<0.05$) de la concentration en sélénium du jaune, de l'albumen et de l'œuf comparativement au témoin.

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Introduction

Due to its participation in antioxidant defense, and in the regulation of redox status selenium plays an important role in the quality of the edible and the breeder egg.
Egg freshness is one of the most important parameters of consumer perception of egg quality. Egg quality decreases during storage. This process is associated with biochemical changes in composition and in the structure of the egg. In experiments conducted in Japan (Wakebe, 1998) the inclusion of selenomethionine (Se-Met) in the layer diet, supplying 0.3 ppm Se/kg feed increased GSH-Px activity in the egg yolk and white as well as egg freshness expressed as Haugh units. In addition, the selenium content of the egg is of interest due to the role of selenium in human health (Rayman, 2000).
In the breeder selenium is of particular importance in the chicken embryo tissues as they are rich in long chain polyunsaturated fatty acids (PUFA) and as a result are very vulnerable to lipid peroxidation. Therefore, Se is a crucial factor in maintaining appropriate antioxidant defense during embryonic development (Surai, 2002).
Hence, it is important in both layers and breeders that the egg contains sufficient selenium. The aim of this study was to determine the effect of an organic selenium source (Sel-Plex®, Alltech Inc.) on the transfer of selenium into the egg of broiler breeder hens under commercial conditions.

Material and Methods

The trials included a total of 30 commercial breeder houses on 15 different farms (Ross 308 and Cobb 500). Two randomized block design trials were conducted including 9 and 6 farms (2 houses per farm), respectively. All flocks were fed diets meeting commercial nutrient specifications. In trial 1 diets were supplemented with Na-selenite (0.2ppm Se) or Sel-Plex® (0.2ppm Se). In trial 2 diets were supplemented with Na-selenite (0.2ppm Se) or a combination of Na-selenite (0.2ppm Se) and Sel-Plex® (0.2ppm Se). Sel-Plex® (Alltech Inc.) is an organic selenium supplement produced by growing yeast in the presence of selenite. After a minimum of 3 weeks supplementation 12 eggs per flock were randomly selected, separated into shell, yolk and albumen, the fractions were pooled and weighed. The concentration of selenium in both the yolk and albumen were analyzed. Selenium was analyzed using hydride generation atomic absorption spectrometry after hydrolysis of the sample with a mixture of nitric/perchloric/sulfuric acid. Data were analyzed as a randomized block design by ANOVA.

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Results and Discussion

In both trials Sel-Plex® led to a significant increase in selenium concentrations in the yolk, albumen and total egg compared to the control. Selenium content of the total egg was 17.43 and 21.30µg (SE=0.20) in trial 1 and 16.29 and 26.10µg (SE=1.14) in trial 2 for the Na-selenite and the Sel-Plex® or combination treatment, respectively. The values of trial 1 are in the same range as values (14 and 22 µg) reported by Paton *et al*. (2000) who were feeding diets with similar selenium supplementation to layers under research conditions. These data indicate that selenium supplementation with Sel-Plex® is an efficient way of enhacing the selenium content of the egg. Antioxidative status of the egg is important during storage and also for embyonic development (Surai, 2002). Dietary antioxidant supplementation in general and organic Se supplementation in particular should be considered to be major protective measures for maintaining high reproductive characteristics in poultry.

Table 1: Selenium content of yolk, albumen and total egg from hens fed Na-selenite or Sel-Plex® (Trial 1)

Treatment	n	Yolk		Albumen		Egg
		ng/g	µg (total)	ng/g	µg (total)	µg (total)
Selenite	9	595.1	13.2	125.3	4.25	17.43
Sel-Plex®	9	638.3	14.3	206.6	7.00	21.30
SE		5.93	0.18	3.92	0.13	0.20
P		< 0.001	< 0.005	< 0.001	< 0.001	< 0.001

Table 1: Selenium content of yolk, albumen and total egg from hens fed Na-selenite or are combination of Na-selenite and Sel-Plex® (Trial 2)

Treatment	n	Yolk		Albumen		Egg
		ng/g	µg (total)	ng/g	µg (total)	µg (total)
Selenite	6	549.3	12.2	113.3	4.09	16.30
Sel-Plex®	6	722.0	16.9	222.3	8.08	24.10
SE		40.1	0.87	9.94	0.28	1.14
P		< 0.05	< 0.05	< 0.001	< 0.001	< 0.005

Conclusions

Substitution or supplementation of inorganic selenium with Sel-Plex® significantly increased egg selenium content under commercial conditions.

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Significant increases were seen in both the yolk and albumen fractions of the egg.

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Session 12
Functional properties of egg and egg products

FUNCTIONAL PROPERTIES OF EGG YOLK

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Keywords : egg yolk, emulsification, gelation

Abstract

Emulsification, emulsion stabilization, foaming, coagulation and gelation are well-known functional properties of yolk in dispersed food systems (emulsions, gels or solid foams). Due to yolk's complicated composition and to organization of its constituents into supermolecular structures, the picture regarding the way yolk functions is still obscure. Recent research findings point out to the importance of LDL micelar constituent apoproteins and secondarily of granular ones in determining the emulsifying behaviour of yolk. Apolipoproteins of LDL micelles also appear to play a crucial role in yolk coagulation and gelation, which determine structure formation of products such as creams and cakes. Although physical forces operating between yolk constituents are important interactions in determining structure and rheological properties of yolk containing products, disulfide bond formation also appears to play a key role in yolk gel network development.

Résumé

Emulsification, stabilisation d'émulsion, pouvoir moussant, coagulation et gélification sont des propriétés fonctionnelles bien connues du jaune d'œuf dans des systèmes alimentaires dispersés comme les émulsions, les gels ou les mousses. A cause de sa composition complexe et de l'organisation des constituants du jaune d'œuf en structures supermoleculaires, la compréhension des fonctionnalités du jaune d'œuf est encore obscure. Des recherches récentes indiquent l'importance des constituants des apoprotéines des LDL micellaires et deuxièmement celles des granules, dans la détermination du comportement émulsifiant du jaune d'œuf. Les apolipoprotéines des micelles LDL semblent également jouer un rôle crucial dans la coagulation et la gelation du jaune d'œuf, ce qui détermine la formation de structure de produits tels que les crèmes et les gâteaux. Bien que les forces physiques existant entre des constituants

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du jaune d'œuf sont des interactions importantes dans la détermination de la structure et des propriétés rhéologiques des produits contenant du jaune d'œuf, la formation des ponts disulfures semble jouer un rôle clé dans le développement de gels à base de jaune d'œuf.

Introduction

Eggs are known among the best foods providing a number of valuable nutrients to human diet such as proteins of high biological value, phospholipids, essential fatty acids, vitamins and minerals. Shell eggs are made up of shell (8-10%), albumen (56-61%) and yolk (27-32%).

The food industry very often utilizes egg products such as egg white, egg yolk or whole egg which may be marketed in the form of refrigerated, frozen or spray-dried form. Commercially available yolk may contain up to 15-20% albumen due to adherence of egg white to the vitelline membrane during the separation of the intact yolk (Powrie and Nakai, 1985). Egg products are employed in the manufacture of foods such as mayonnaise and salad creams, bakery products and cakes, noodles, baby foods, creams etc. not only because of their undoubted nutritional quality and the highly appreciated by the consumer organoleptic properties but also because egg constituents are very well known for their excellent functional properties that are necessary to prepare a physicochemically stable product with optimum rheological and textural properties.

Yolk, in particular, is a food ingredient exhibiting excellent emulsifying and emulsion-stabilizing properties and in products such as cakes or creams it may act simultaneously as an emulsifier and as a structure-forming material (Kiosseoglou, 1989). It is important, therefore, to know the way the yolk constituents function in various products in order to control the parameters which influence its functionality in the most beneficial way for the production of foods acceptable by the consumer. This task is not an easy one since our knowledge regarding yolk functionality is rather limited compard to other food ingredients due mainly to the very complex nature of this material. Egg yolk constituents are organized into large spheres, granules and low density lipoprotein micelles while a number of globular proteins called livetins are dispersed in yolk plasma. This structure is the result of a complex mixture of proteins and lipids combined in a fat dispersion in a water phase where the apolipoproteins act as emulsifying and stabilizing agents of this physicochemical system. The functionality of yolk constituents can, therefore, be viewed as the disorganization of yolk particles in presence of oil droplet surfaces or as a result of heating leading to adsorption and spreading at o/w interfaces or to gel network structure formation, respectively.

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Yolk constituents in emulsions

Emulsion formation and stabilization by proteins are determined to a large extent by the ability of these macromolecules to adsorb, unfold and rearrange at the o/w interface resulting in reduction of interfacial tension followed by molecular interactions, which lead to the development of a strong and cohesive adsorbed protein film around the oil droplets. Although this theory may describe in general lines the adsorption behaviour of a typical protein from its solution, the situation becomes far more complicated in the case of egg yolk. The yolk constituent proteins differ considerably in structure and flexibility and most of them are organized into supermolecular structures. Furthermore, other surface-active constituents also exist in yolk, i.e. phospholipids and cholesterol, which may adsorb at the emulsion o/w interface together with the yolk proteins resulting in the formation of a mixed adsorbed membrane around the oil droplets.

The role of yolk constituents in adsorbed film formation and emulsion stabilization has not up to now been established as a result of yolk's complicated structure. Yet, some progress has been made in the last two decades starting with the pioneering work of Shenton (1979) who was the first worker to investigate the composition of adsorbed interfacial membrane material of o/w emulsions stabilized by an egg – milk protein mixture. Based on research findings regarding the chemistry of biological materials with similar structure, i.e. serum LDL and HDL, Shenton suggested that yolk protein constituents differ in their ability to adsorb and penetrate the interfacial film with the apoliporopteins of LDL being more surface-active as a result of their high molecular flexibility and hydrophobicity. Shenton's theory about yolk protein adsorption were exploited by Kiosseoglou and Sherman (1983a, b) in their investigation about the role of yolk in stabilizing and modulating the rheological properties of salad dressing emulsions. Major contributions to our understanding of yolk functionality in emulsions are research works conducted in recent years by Mine (1998a, b), and Anton and his co-workers (Anton and Gandemer, 1999; Le Denmat et al., 2000; Anton et al., 2000, 2001) who studied the emulsifying and adsorption behaviour of isolated yolk fractions like LDL micelles and granules, as well as of whole yolk as influenced by parameters such as pH and NaCl.

The most important conclusions derived so far regarding yolk functionality in emulsions are:

a) Yolk proteins exhibit marked differences in their ability to adsorb at o/w interfaces depending on molecular charge and flexibility, extent of supermolecular structure disorganization, protein dimerization and solubility and homogenization conditions.
b) LDL apoliporoteins play a dominant role in emulsion formation and stabilization and emulsions prepared with yolk plasma resemble in many aspects those of egg

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yolk. Granule proteins, however, may also contribute to emulsion interfacial film formation depending on factors such as pH, NaCl content and degree of granular structure disorganization.

c) Yolk neutral lipids coalesce with the emulsion oil phase while surface-active lipids of yolk are partially adsorbed at the o/w interface, which is, however, dominated by yolk apolipoprotein constituents.
d) Other egg proteins (albumen) are not able to adsorb at the interface when yolk is present, due to their more compact globular structure, but nevertheless may influence the rheological properties of emulsions, indicating that some kind of interaction takes place with the adsorbed yolk proteins.
e) The rheological properties of yolk-based emulsions depend to some extent on the state of supermolecular structure disorganization, the molecular charge and the interaction of yolk proteins with other polymeric materials present in the system (i.e. polysaccharides).

Yolk emulsions are notable for their exceptional stability against droplet coalescence and commercially prepared salad dressings are very difficult to study under normal storage conditions. This is attributed to the viscoelastic character of the adsorbed interfacial film that may resist deformation forces leading to rupture and droplet coalescence (Kiosseoglou and Sherman, 1982; Melnikov, 2002). Although, however, yolk is a very effective emulsion stabilizer with respect to droplet coalescence, its ability to stabilize emulsions against creaming, as is the case for all protein emulsifiers, is rather limited. Concentrated o/w emulsions such as mayonnaise are quite stable due to close packing of oil droplets that allows no space for structure reorganization and phase separation. In the case of less concentrated emulsion systems, creaming destabilization may become a problem when the products are stored for long periods and it is a common practice to use polysaccharides in order to enhance emulsion stability.

Yolk constituents may contribute to emulsion stabilization against creaming depending on pH and particle structure disorganization. Yolk proteins adsorbed at the interfaces appear to influence the rate of creaming through steric or bridging flocculation effects. As was pointed out by Le Denmat et al. (2000) the stability of yolk emulsions depends to a large extent on steric repulsions due to adsorbed apolipoproteins of LDL. Granular proteins although they represent a significant proportion of adsorbed proteins seem to be less effective in droplet stabilization against creaming. A recent investigation conducted in our laboratory, however, indicated that creaming stability at neutral pH is heavily dependent on the degree of granular structure disorganization. Thus, when granules are completely disorganized as a result of 0.6M NaCl addition, their apoproteins adsorb as isolated molecules and in this way they probably spread at the

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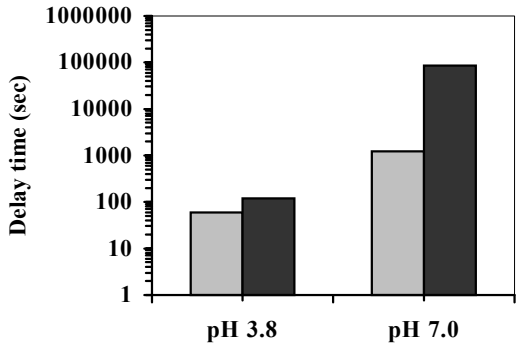

Figure 1. Mean delay time of liquid yolk – based emulsions (10% corn oil, 1% yolk) in absence ( ) or in presence of 0.6M NaCl (ˇ).

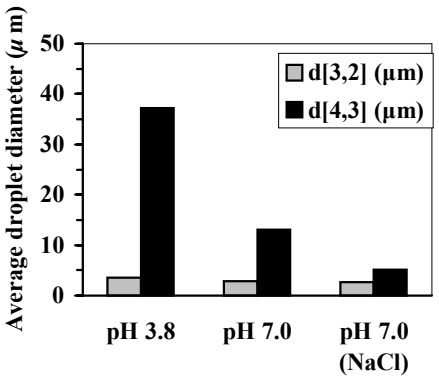

Figure 2. Influence of pH and/or NaCl addition on average droplet diameter ($d_{[3,2]}$ or $d_{[4,3]}$) of yolk-based emulsions (10% corn oil, 1% yolk) or in presence of 0.6M NaCl.

interface obtaining favourable conformations which may maximize steric repulsive forces and enhance creaming stability (Fig. 1). When granules are adsorbed as intact or partially disorganized particles (pH 7.0, low NaCl content) or in the non-soluble state (pH 3.8), interactions between the oil droplets may take place as a result of adsorbed granules leading to a form of bridging flocculation (Fig. 2) and emulsion destabilization.

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Yolk proteins in gel network formation

The characteristic textural properties of food products such as omelets, cakes, creams etc, are indebted to a large extent to egg protein coagulation and/or gelation following heating and protein denaturation. Both the albumen and yolk fractions have the ability to form gel-networks when heated at temperatures beyond 70°C. Stirred yolk, in particular, is capable of forming very firm gels with a hard, cohesive and rubbery texture depending on pH and NaCl concentration (Woodward, 1990). Yolk solutions at pH 7.0 take longer to gel than at pH 3.0 or 5.0 (Anton et al., 2001). The rapid coagulation at acid pH was attributed to the shift in isoelectric conditions of apolipoproteins of LDL in presence of 0.55M NaCl resulting in a coagulum-type gel being formed at acidic pH. At neutral pH the gel network was more organized exhibiting a higher final G' value.

Apoproteins of LDL appear to play a key role in yolk gelation. As was pointed out by Anton et al. (2001) the gelation of yolk solutions follows a similar pattern compared to yolk plasma while disorganized yolk granules are less effective in gel network formation. Granule gelation is a much slower process and the resulting gels exhibit a lower gel strength. According to Nakamura et al. (1982) gels prepared with LDL in the absence of NaCl exhibit a minimum in rigidity at pH values between 6.0 and 7.0 indicating that ionic forces operating between interacting proteins are indirectly involved in gel network formation leading to random interaction pattern and an inhomogeneous gel of low rigidity. Although, however, physical forces should be partly responsible for yolk protein interactions during the formation of a gel network, a recent investigation conducted in our laboratory (unpublished results) indicated that covalent disulfide interaction forces between yolk protein molecules could play a decisive role in yolk gelation. As shown in Fig. 3a the critical yolk concentration to prepare a gel in presence of N-ethylmaleimide, a –SH group blocking reagent, is 15%, well above the concentration of 5% required in its absence. Furthermore, the compressive strength of gels is dramatically reduced when disulfide bond formation between yolk protein molecules is prevented.(Fig. 3b). Following protein denaturation sulfhydryl groups previously buried in the interior of lipoprotein of yolk may lead to disulfide bond formation either through oxidation or sulfhydryl-disulfide exchange interactions. Both the HDL and the LDL of yolk apoproteins contain sulfhydryl groups and as was pointed out by Nguyen and Burley (1980) in the apoproteins of LDL the balanced distribution of sulfhydryl and disulfide groups may be responsible for the appearance of extensive aggregation phenomena in heated egg yolk through sulfhydryl – disulfide interchanges.

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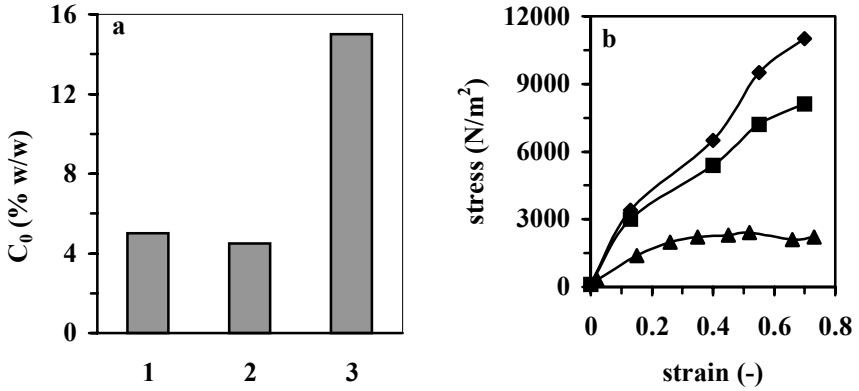

Figure 3. (a) Effect of presence of NaCl or N-ethylmaleimide on minimum yolk solids concentration, C_0, for gel formation by liquid yolk Key: 1, control; 2, 0.6M NaCl; 3, 0.006M N-ethylmaleimide.

(b) Stress-strain curves for liquid yolk gels (30% in yolk solids) in presence of N-ethylmaleimide. Key: (◆) control; (■) 0.02M and (▲) 0.06M N-ethylmaleimide.

The gelation behaviour of yolk may also be markedly influenced by the degree of disorganization of yolk particle structure imposed as a result of processes such as pasteurization, freezing, drying or cholesterol extraction. Spray-dried yolk is a well known commercially available product widely used as ingredient in many egg-based foods. Following rehydration of spray-dried yolk a «weak» gel structure results at a relatively low temperature and in this way it differs from a liquid yolk dispersion or a typical dehydrated protein solution such as egg albumen indicating that the dissorganized yolk particles can interact through weak physical forces and the dispersion-like structure of spray-dried yolk may lead to a dominant elastic response at 35°C (Fig. 4). Following cooling to 5°C and then heating again to 35°C a symmetric increase and decrease in the viscoelastic moduli was observed which may be attributed to the network strengthening during cooling as a result of an increase in the number of reversible physical interactions. When the samples are heated beyond 35°C the dramatic increase in the structure formation should be attributed to further protein denaturation leading to a gel structure of high stiffness and limited frequency dependance of storage modulus (Paraskevopoulou and Kiosseoglou, 2000).

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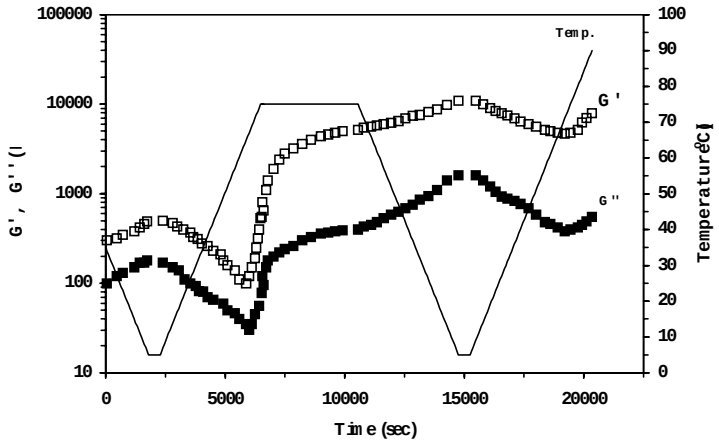

Figure 4. Storage modulus G' ( ) and loss modulus G'' (ν) variation as a function of time of spray-dried yolk (1.5Hz, yolk solids content 35%).
From Paraskevopoulou and Kiosseoglou (2000).

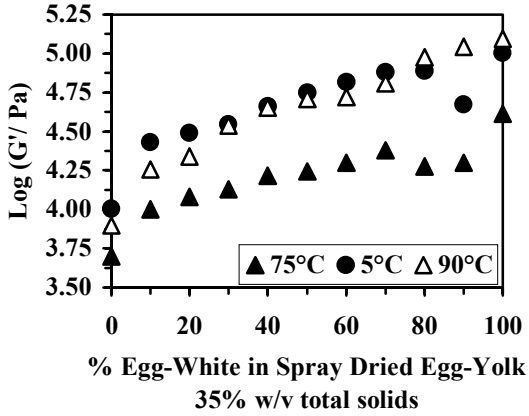

Figure 5. Storage modulus G' as a function of the composition of the mixture containing spray dried yolk and egg white (Total solids content 35%; Frequency 1.5Hz; Strain 0.2%).
From Paraskevopoulou et al. (2000).

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A common practice in the preparation of egg–containing food products is to use mixtures of yolk with egg white in the form of whole egg. The presence of albumen in this case may affect the gelation behaviour of yolk. It appears that following heating of the yolk/albumen mixture a composite gel is formed where, for a typical yolk/albumen solids proportion found in whole egg, yolk constitutes the continuous phase while egg white stiffens the gel structure by acting as a discontinuous filler phase. Only when the egg white content exceeds 70% in solids in the mixture, phase inversion takes place resulting in the weaker egg white becoming the continuous phase at temperatures up to 75°C (Fig. 5). When heating, however, mixtures containing more than 70% egg white solids at 90°C, networks with a higher stiffness appears compared to the egg yolk-continuous gels. This is attributed to further albumen protein denaturation at temperatures above 80°C resulting in the reinforcement of the continuous egg white gel network (Paraskevopoulou et al., 2000).

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OVOTRANSFERRIN DENATURATION AT AIR-WATER INTERFACE

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Keywords : ovotransferrin, air-water interface, structure

Abstract

Egg white proteins are well known for their sensitivity to heat treatments, shearing or exposition to interfaces. Effects of interfaces on ovotransferrin structure have not been extensively studied. This protein is in fact extremely denatured at the air-water interface. Its tertiary structure is strongly modified: the aromatic residues are exposed (significant red shift of 1 nm in intrinsic fluorescence) and the surface hydrophobicity is increased by 30 %. Its secondary structure is also altered, as shown by far UV CD measurements: 33 % of α-helix is lost in favor of ß-sheet (+96%). When mixed with ovalbumin, no significant changes in its secondary structure occurred but modifications of the tertiary structure suggested the aggregation of the protein: the fluorescence intensity is decreased by 55% and surface hydrophobicity by 40%. When mixed with lysozyme, ovotransferrin did not seem to be affected by the air-water interface: no significant changes of tertiary or secondary structures.

Résumé

Les protéines de blanc d'œuf sont réputées pour leur sensibilité aux traitements thermiques, cisaillements et interfaces. Les effets de ces dernières sur les protéines n'ont pas été beaucoup étudiés, en particulier pour l'ovotransferrine. Celle-ci est en fait très dénaturée après contact avec l'interface air-eau. Sa structure tertiaire est fortement modifiée : exposition des résidus aromatiques (red shift significatif d'1 nm en fluorescence intrinsèque) et augmentation de 30% de l'hydrophobicité de surface. Sa structure secondaire est également altérée, comme le montrent les mesures de CD en UV lointain : perte de 33% des hélices α en faveur des feuillets β (+96%). En mélange avec l'ovalbumine, aucun changement significatif dans la structure secondaire n'est observé. En revanche, les modifications de structure

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tertiaire supposent l'agrégation de la protéine : diminution de l'intensité de fluorescence de 55% et de l'hydrophobicité de surface de 40%. En mélange avec le lysozyme, l'ovotransferrine ne semble pas affectée par le contact avec l'interface air-eau : pas de changements significatifs des structures tertiaires ou secondaires.

Introduction

Egg white proteins have great functional properties that egg product manufacturers try to protect. But these properties are often impaired after industrial egg white processing that involves many technological treatments, such as mechanical, thermal treatments and large interfacial area creation (Britten et Lavoie, 1992; Croguennec *et al.*, 2002). Contact with interfaces is often considered as to be the less denaturing. If mechanisms of protein diffusion to an air-water interface have been extensively studied, including for ovotransferrin (Damodaran *et al.*, 1998), structural modifications are not completely clarified, especially for ovotransferrin. This protein is renowned for its flexibility relating to its biological properties (Jeffrey *et al.*, 1998). It is also the most heat-labile of the egg white proteins (Matsuda *et al.*, 1981). These are the reasons why we tried in the present study to highlight ovotransferrin behavior at the air-water interface either in the absence or presence of two other major egg white proteins, ovalbumin and lysozyme.

Material and Methods

Ovalbumin and ovotransferrin were extracted from hen egg white by anion exchange chromatography according to Croguennec *et al.* (*2000-2001*). Lysozyme was obtained from Ovonor (Annezin les Béthune, France). Proteins were mixed in the same proportions as in egg white.
Air-water interfaces were created with a bubbling column PM 930 (Grosseron, St Herblain, France) from 1% protein solutions, pH 7.0. At the top of the column, foam was withdrawn via a plastic tube. The drained liquid was separated from the foam by decanting and then reintroduced twice in the bubbling column in order to obtain more foam. The foam was collapsed by centrifugation at 3000 g for 10 min at 20°C. The "foam skeleton" obtained was solubilized in 67 mM phosphate buffer, pH 7.0.
Native protein solutions and solubilized foam skeleton were analyzed by gel permeation chromatography (TSK gel G3000 SWXL, Tosoh Biosep, Tokyo, Japan).

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For further structure analysis, ovotransferrin and lysozyme were separated by gel permeation chromatography whereas ovotransferrin and ovalbumin were separated by anion exchange chromatography (Q-Hyper D10, Biosepra, Villeneuve-la-Garenne, France). Each fraction was collected, lyophilized and solubilized in 67 mM phosphate buffer, pH 7.0 to the same concentration as the native solutions.

Intrinsic fluorescence measurements were performed (spectrofluorimeter LS50B, Perkin Elmer, Norwalk, USA) at pH 7.0 after excitation at 280 nm or 295 nm. Emission spectra were registered between 305 and 415 nm. The slope of the relative fluorescence intensity versus protein concentration (3 concentrations tested) was then used as an index of the protein intrinsic fluorescence.

Quenching fluorescence measurements were performed after excitation at 295 nm to minimize interference from tyrosyl residues. L-tryptophan solutions were used as the reference. Quenchers used were acrylamide (0 to 67 mM), cesium chloride (0 to 0.67 M) and potassium iodide (0 to 0.67 M). The quenching constant was expressed by the Stern-Volmer equation:

$$F_0 / F = 1 + K_q[Q]$$

where F_0 and F are the fluorescence intensities in the absence and in the presence of the quencher, respectively, [Q] is the quencher concentration, and K_q is the quenching constant. The percentage of tryptophyl residues accessible to quenchers was calculated as follows:

$$\text{Trp \%} = [K_q(\text{sample}) / K_q(\text{L-trp})] \times 100$$

Measurement of surface hydrophobicity was carried out using the fluorescence probe ANS as suggested by Kato and Nakai (*1980*). The slope of the fluorescence intensity versus protein concentration was used as an index of the protein surface hydrophobicity (PSH). The relative surface hydrophobicity (RSH) was calculated as follows:

$$\text{RSH (\%)} = (\text{PSH of sample}) \times 100 / (\text{PSH of control})$$

The native solution sample was used as a control.

CD spectra were obtained using a CD 6 spectropolarimeter (Jobin-Yvon, Paris, France). Protein concentrations were 0.77 $g.L^{-1}$. Far UV CD spectra were recorded from 180 to 250 nm with a 0.02 cm light path. Near UV CD spectra were recorded from 250 to 330 nm with a 1 cm light path. Each spectrum was the average of three scans. CD spectra were expressed in terms of molar ellipticity $[\theta_\lambda]$:

$$[\theta_\lambda] = (3300 \times \Delta A_\lambda) / (C \times d)$$

with $[\theta_\lambda]$, the molar ellipticity at the wavelength λ expressed in $deg.cm^2.dmol^{-1}$; ΔA_λ, the difference of a right and left-circular polarized light of equal intensity and of the same wavelength λ; C, the mean residue concentration when the far UV CD spectrum was reported and the protein molarity in the near UV region; d (cm), the light path. α-helix, β-sheet, β-turn and random coil structures were determined from the far UV CD spectra using CD spectra deconvolution software described by Bohm *et al. (1992)*.

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Results and discussion

All results given are expressed as the difference observed between ovotransferrin before and after contact with the air-water interface.

Gel permeation chromatography.

This analysis revealed that lysozyme was preferentially retained in the foam phase when mixed with ovotransferrin in agreement with what observed Poole *et al.* (1984). It is likely that with its small size, lysozyme was the first to reach the interface and was then stuck by ovotransferrin. On the other hand, in the ovotransferrin-ovalbumin mixture, the proportion of ovotransferrin increased by 8% in the foam fraction. Two reasons could be given: either ovotransferrin was preferentially adsorbed at the air-water interface, or ovalbumin was not as well solubilized as ovotransferrin because of its ability to aggregate via disulfide bonds. This second hypothesis is the more likely because ovalbumin is renowned to be one of the first to reach the interface (Damodaran *et al.*, 1998). Whatever, these variations of ratio required us to separate out the proteins in native and foam solutions (cf. material and methods) to enable the structural analyses and the comparison with ovotransferrin alone in solution.

Tertiary structure modifications.

For ovotransferrin alone in solution, contact with air-water interface induced a 0.8 nm red shift (figure 1) and a decrease in fluorescence intensity (-33%) (data not shown). Tryptophyl residues were more accessible to acrylamide and iodide (figure 2). Surface hydrophobicity increased by 40% (figure 3) and near UV CD spectrum was widely modified (figure 4). Thus, after contact with the air-water interface, ovotransferrin exposed its aromatic and hydrophobic residues assuming a deployment of the molecule. This agreed with Acton *et al.* (1990) who showed that ovotransferrin molecular flexibility enabled conformational alteration in the interfacial region.

When mixed with ovalbumin, shifts observed in intrinsic fluorescence were more significant (figure 1). Tryptophyl residues were much more accessible to acrylamide, iodide but above all cesium (figure 2). This assumed large modifications around tryptophyl which were close to positively charged residues. Decrease in fluorescence intensity was also higher (-54%) (data not shown). Modifications in near UV CD spectrum were quite similar to that observed for ovotransferrin alone in solution (figure 4). The great difference was for surface hydrophobicity which decreased by 40% (figure 3). This last result suggested an aggregation of the protein which probably followed the deployment of ovotransferrin highlighted by the previous results. This aggregation was probably partly due to the free sulfhydryl groups of ovalbumin: once the interface reached,

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ovotransferrin denatured, exposing its disulfide bonds free for intermolecular sulfhydryl-disulfide exchanges. This phenomenon was already observed by Xu *et al.* (1998) and Watanabe *et al.* (2000) during the heat treatment of ovotransferrin in the presence of ovalbumin.

When mixed with lysozyme, shifts observed in intrinsic fluorescence were of the same nature as the ones observed in mixture with ovalbumin (figure 1). Moreover, tryptophyl residues were not significantly (student test, $P \leq 0.05$) more accessible to quenchers after contact with the air-water interface than before (figure 2). In the same way, surface hydrophobicity was not significantly modified (figure 3) and near UV CD spectrum was not so different from the native molecule one (figure 4). Ovotransferrin seemed to be protected by lysozyme from the interface denaturation. This reinforced the hypothesis mentioned above assuming that lysozyme was the first to reach the interface, preventing by this way ovotransferrin from denaturation.

Secondary structure modifications.

These changes were estimated via far UV CD measurements (figure 5). Differences observed between spectra of native ovotransferrin alone and in mixtures were essentially due to the separation via chromatography (data not shown), as well as for near UV CD spectra (figure 4). Ovotransferrin alone in solution underwent the most important changes in secondary structure. Actually, a significant loss of α-helix structures (-33%), and gains of β-sheet (+96%), β-turn (+13%) and random coil (+21%) structures were observed ($P \leq 0.01$).

When mixed with ovalbumin, ovotransferrin lost 33% and 6% of α-helix and β-turn structures, respectively, and gained 23% of β-sheet structures ($P \leq 0.05$). Structural modifications suffered by ovotransferrin seemed then quite different in the presence of ovalbumin, probably due to aggregation previously mentioned.

In the presence of lysozyme, secondary structure modifications were not statistically different, limited to a loss of 3% of β-turn structures ($P \leq 0.05$). This result confirmed the lesser ovotransferrin denaturation in the presence of lysozyme.

Ovotransferrin denaturation at the air-water interface is very dependant from the environment of the protein. Alone in solution, this protein is highly denatured at the interface. Mixed with ovalbumin, denaturation is combined with aggregation whereas mixed with lysozyme, denaturation is weakened. Thus, extrapolations to complex protein solutions such as egg white from results obtained with pure protein in solution are hardly possible.

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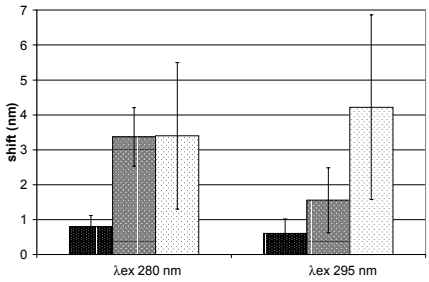

Figure 1. Shifts observed in intrinsic fluorescence after excitation at 280 or 295 nm between native and foamed ovotransferrin alone ( ) or mixed with ovalbumin ( ) or lysozyme ( ).

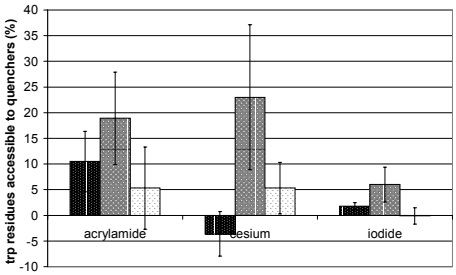

Figure 2. Variations in the percentage of tryptophyl residues accessible to quenchers between native and foamed ovotransferrin alone ( ) or mixed with ovalbumin ( ) or lysozyme ( ).

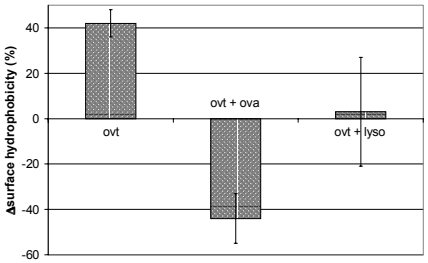

Figure 3. Modifications of surface hydrophobicity between native and foamed ovotransferrin.

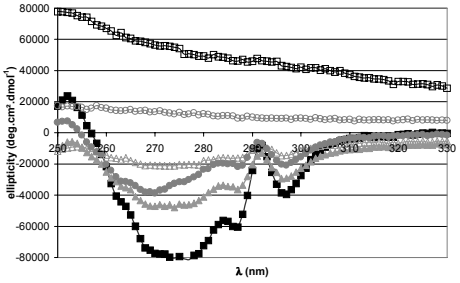

Figure 4. Near UV CD spectra of ovotransferrin alone (native ■ or foamed □) or mixed with ovalbumin (native ● or foamed ○) or lysozyme (native ▲ or foamed Δ)

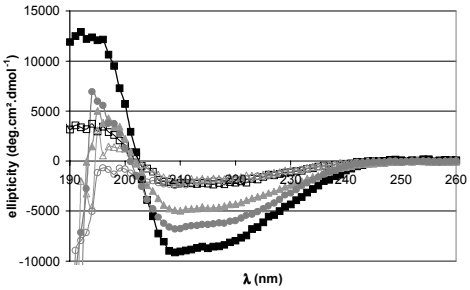

Figure 5. Far UV CD spectra of ovotransferrin alone (native ■ or foamed □) or mixed with ovalbumin (native ● or foamed ○) or lysozyme (native ▲ or foamed Δ)

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EGG PRODUCT QUALITY TRAITS FOR FOOD INDUSTRY

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Keywords: egg products, functional properties, hygienic quality, organic acids, uracil

Abstract

Seventeen commercial whole egg products were analysed for total bacterial count, proximate chemical composition, legal chemical indices (3OH-butyric, succinic and lactic acids), uracil, whipping capacity, and baking performance in a high-ratio cake. All commercial whole egg products were in compliance with the law microbial requirements, however the presence of illegal levels of organic acids highlighted the use of raw eggs of poor hygienic quality for the manufacture of some samples and, in one case, also the use of incubator reject eggs. Functional quality of samples was effectively discriminated by whipping capacity. Results were statistically elaborated by principal components and clustering analyses in order to highlight any correlation between hygienic and functional quality of egg products.

Résumé

Dix-sept produits du commerce à base d'œuf entier ont été analysés pour la contamination microbienne, la composition chimique de base, les indices chimiques prévus par la législation (acides 3OH-butyrrique, lactique et succinique), l'uracile, la capacité foisonnante et les propriétés fonctionnelles dans un pain de Gênes. Tous les produits présentaient un niveau de contamination microbienne dans les limites prévues par la loi. Pour les acides quelques échantillons dépassaient le limites légales témoignant l'utilisation de matière première de mauvaise qualité hygiénique et dans un cas d'œufs de rebut de couvoir. Les qualités fonctionnelles des échantillons peuvent être discriminées sur la base des capacités foisonnante. Les résultats ont été traités statistiquement afin de mettre en évidence toutes les corrélations possibles entre qualité hygiénique et propriétés fonctionnelles.

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Introduction

A recent Dutch survey identified five critical purchasing factors for buyers of egg products (Tacken *et al*., 2003). The buyers ranked the factors in the following order of decreasing importance: microbial quality, price, producer, hen housing system, country of origin.

The first three ranking factors are certainly the most important in affecting buyers choice. Being safety the most compelling requirement for food industry, egg products leaving production factories regularly meet microbial restrictions provided by law (Table 1). Actually, all egg products are sanitized through a pasteurization treatment able to comply with total bacterial count (M = 10^5 per g or ml) and other law limitations for salmonellae, enterobacteriaceae, and staphylococci in egg products (European Commission, 1989). Thus, being safety guaranteed by pasteurization, the buyers purchase from producers offering the cheapest price. This choice often causes nasty experiences, since low-cost egg products may not show the expected functional/technological properties (i.e. foaming, emulsifying, gelling properties). Trust in the producer becomes then important, and buyers select few reliable producers which guarantee egg product supplies with constant technological quality. Interestingly, the technological quality of egg products was not included among the five purchasing factors reported in the survey, even though it may cause on-processing and post-processing troubles to foodstuffs.

Foams, emulsions, baked products, and egg pasta are the foods most sensitive to variations of egg product functional properties. Sometimes it is possible to correct functionality deficiencies with weight increase of the egg ingredient, directly in food manufacturing. In other cases, defects may become evident only during food storage: e.g. refrigerated mousse-desserts may loose volume during distribution and retailing.

The use of egg products with poor functional properties leads anyway to economic losses because of: higher production costs as a consequence of increased dosage of the egg ingredient; downgrading of the defective finished food (e.g. a poorly whipped sponge cake); withdrawal from the market of the already distributed food.

This research is a preliminary study to identify the reasons for variability in egg products functional properties. The focus is not on the effect of well-known compositional or technological factors (e.g. albumen to yolk ratio, homogenization, pasteurization) (Yang and Baldwin, 1995; Rossi, 1999), but rather on factors related with the hygienic quality of the eggs used as raw material for egg products manufacture.

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Material and Methods

Seventeen commercial whole egg products were analysed for proximate chemical composition, legal chemical indices (3OH-butyric and succinic acids by enzymatic kits; lactic acid by HPLC), uracil (Hidalgo et al., 2003), whipping capacity (Barone, 2001) and baking performance (cake volume) in a high-ratio cake (Rossi et al., 2001). The results were statistically elaborated by principal components and clustering analyses to spot correlations between hygienic and functional quality of egg products.

Results and discussion

All commercial whole egg products were in compliance with law microbial requirements; however the presence of illegal levels of organic acids highlighted the use of raw eggs of poor hygienic quality for the manufacture of samples N, O, and P (Figure 1). In sample O, the content of 3OH-butyric acid above the legal limit indicates also the fraudulent use of incubator reject eggs. However, also samples K, L, M, and Q, due to their detectable uracil, may be considered as obtained from spoiled eggs. The higher reliability and sensitivity of uracil with respect to other spoilage indicators (lactic and succinic acids) has been discussed in a paper in press (Hidalgo et al., 2003).

Figure 2 reports whipping capacity of whole egg samples as percentage volume increase (overrun %). A high variability is observed, ranging from a minimum overrun value of 181% to a maximum of 516%. This variation may be explained by differences in the content of the functional agent, i.e. proteins, which ranged from 10.4 to 13.1 g/100g in the whole egg products analyzed. Actually, a significant direct correlation ($p<0.01$) was observed between protein content and whipping capacity. Whipping capacity was also directly correlated ($p<0.001$) to baking performance. This means that functional quality of whole egg products can effectively be discriminated by whipping capacity.

Comparing the values reported in Figure 1 and Figure 2, it is evident that all samples with detectable uracil values (with the exception of sample N) have poor or medium whipping capacities. Thus, the influence of raw egg hygienic quality on egg products functional properties can be argued. Poor whipping capacity may be ascribed to microbial enzymatic activities, protease in particular, affecting protein structure, and thus functional properties. During egg product manufacture, pasteurization effectively reduces microbial population and mostly inactivates enzymes, but protein damages occurred before pasteurization are irreversible.

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However, other factors mainly linked to technological processes should also be considered as potential source of variation for egg product functional performance. In fact, also egg products E, G, and J show poor whipping capacities, notwithstanding their organic acids and uracil negligible values, which indicate that sound raw eggs were used for their production.
Principal components and clustering analyses were carried out on the following six variables: succinic, lactic, and 3OH-butyric acids (legal variables), overrun and baking performance (functional variables), and dry matter (compositional variable). Through multivariate analysis, industrial whole egg samples (after exclusion of sample N) were classified into three groups of different quality (Table 2). The results stress the importance of raw egg good hygienic quality to obtain egg products with satisfying functional performances (grade A). However, some samples obtained from sound raw eggs have poor functional properties (grade B), whilst spoiled raw eggs always produce egg products with poor functional properties (grade C). This means that raw egg good hygienic quality is necessary but not sufficient for satisfactory functional properties of egg products.

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Table 1. Analytical specifications for egg products (European Commission, 1989).

MICROBIOLOGICAL CRITERIA
- salmonellae*:* absence in 25 g or ml of egg product
- mesophilic aerobic bacteria: M = 10^5 in 1 g or 1 ml
- enterobacteriaceae: M = 10^2 in 1 g or 1 ml
- staphylococci: absence in 1 g of egg product

OTHER CRITERIA
- 3OH-butyric acid: < 10 mg/kg in the dry matter
- lactic acid: < 1000 mg/kg in the dry matter
- succinic acid: < 25 mg/kg in the dry matter

Figure 2. Whipping capacity (expressed as overrun %) of the industrial whole egg products.

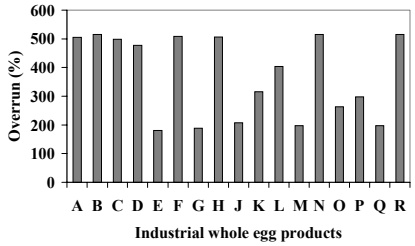

Table 2. Egg product quality grades as identified through principal component and clustering analyses of industrial whole egg products (Barone, 2001).

GRADE	Hygienic quality of raw eggs	Functional Quality
A	+	+
B	+/-	-
C	-	-

+ high; - poor

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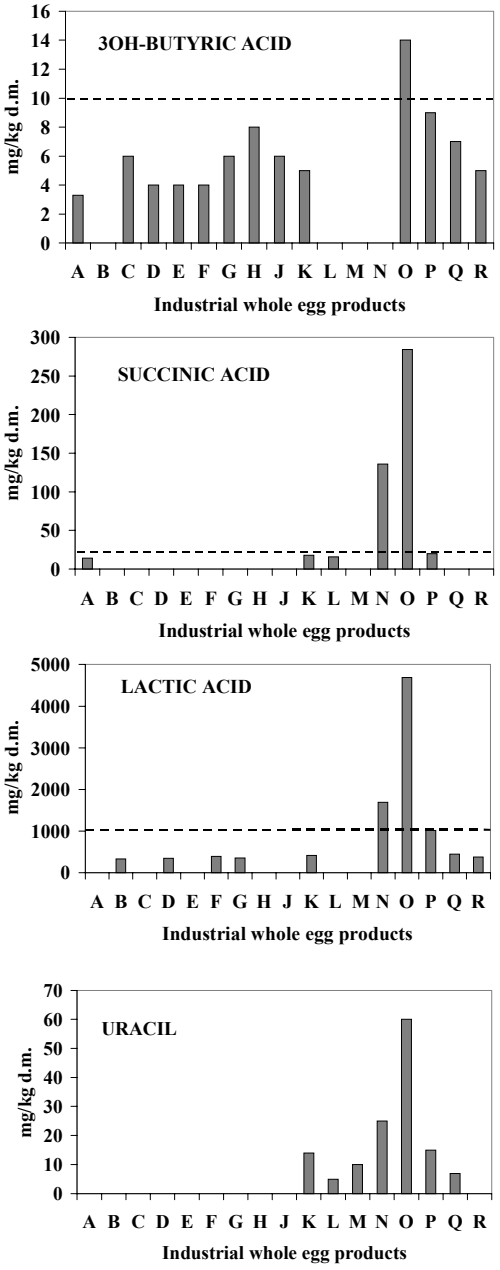

Figure 1.
Organic acids and uracil contents in the industrial whole egg products analyzed. When bars are not shown, values are below the detection limits.

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INGREDIENT EFFECTS ON FREEZE-THAW STABILITY OF PRECOOKED LIQUID WHOLE EGG

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Keywords : whole egg, freeze-thaw, precooked, moisture

Abstract

Added cornstarch and sodium caseinate (0-6%) decreased ($P<0.05$) expressible moisture of precooked and frozen-thawed precooked whole egg products. Modified cornstarch increased expressible moisture whereas egg albumen (dehydrated) had no effect. Soy protein decreased expressible moisture but not to the extent found using sodium caseinate. Sodium caseinate at 3.0 to 4.5% yielded total moisture retention equivalent to that found for freshly cooked whole egg. Pentanal and hexanal contents from GC-MS showed that egg albumen had no effect ($P>0.05$) on these volatiles whereas soy protein acted as a pro-oxidant ($P<0.05$) and sodium caseinate acted as an antioxidant ($P<0.05$). Results demonstrated improvement of freeze-thaw and oxidative stabilities of precooked whole egg with sodium caseinate.

Résumé

L'addition de farine de maïs et de caséinate de sodium (0-6%) diminue l'humidité de produits précuits ou précuits / congelés à base d'œuf entier. La farine de maïs modifiée augmente l'humidité alors que le blanc d'œuf déshydraté n'a pas d'effet. Les protéines de soja diminuent aussi l'humidité mais pas autant que le caséinate de sodium. Celui-ci (3-4.5%) permet une rétention d'eau équivalente à obtenue pour des œufs entiers frais cuits. Les teneurs en pentanal et hexanal montrent que le blanc d'œuf n'a pas d'effet ($P>0.05$) sur ces composés volatils alors que les protéines de soja agissent comme prooxydant ($P<0.05$) et que le caséinate de sodium agit comme antioxydant ($P<0.05$). Ces résultats démontrent l'amélioration de la stabilité vis-à-vis de la congélation et de l'oxydation de l'œuf entier précuit par le caséinate de sodium.

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Introduction

The major problem with precooked frozen foods containing egg as a main ingredient is that they fail to retain water. These products generally have an undesirable texture and appearance due to fluid accumulation within the package (Gossett and Baker, 1983). If ingredients added to eggs are to impart freeze-thaw stability in cooked, frozen egg products, they must interact with the proteins of egg in gelation and bind moisture so that expressible moisture content is reduced or comparable to the same non-frozen product. The objective of this study was to compare moisture binding of cornstarches and various proteins relative to freeze-thaw stability in a precooked whole egg product. Headspace volatiles related to oxidation were also determined.

Materials and Methods

Corn starch, modified cornstarch, sodium caseinate, isolated soy protein and egg albumen were studied at 0-6% addition to fresh broken-out whole liquid egg previously homogenized and adjusted to pH 7.0 with 2M citric acid. Approximately 200 mL of liquid egg was filled into 30 mm diameter cellulosic casings, cooked in water to 72 C internal and cooled for 2-4 h at 4 C. Half of the precooked whole egg product was frozen at -20 C for 7 days, then thawed at 4 C, and the other half was held at 4 C for 7 days.

Total moisture was determined by oven drying using procedure 925.340 (AOAC, 1990). Expressible moisture content was determined using the centrifugal force procedure of Jauregui et al. (1981) as modified by Earl et al. (1996).

Headspace volatiles were determined for the fresh liquid whole egg and precooked non-frozen and precooked frozen-thawed egg products using a Tekmar 7000 Headspace autosampler interfaced to a Hewlett-Packard Model 5890 II capillary gas chromatograph. Samples (4.5 g) were sealed in a 9 mL vial, heated at 100 °C for 30 min, and automatically injected onto the head of a 30 m DB-5 capillary column (0.32 mm i.d., J&W Scientific) maintained at -30 °C. The GC column temperature was programmed from 30-220 C at 4 °C/min. Flame ionization detector signals were stored and integrated using a HP Chemstation. Peak identification was based on retention time of authentic reference standards.

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Results and discussion

The pH of freshly broken whole egg was initially adjusted to pH 7.0 to insure that cooked products would have a pH of less than 8.2 to prevent green-gray discoloration promoted by FeS formation. Mean pH of freshly cooked whole egg products over all ingredient types and levels was 8.03 (Table 1) with a range of pH of 8.24-7.62. With a 0.82 pH unit spread at a mean product pH value of 8.0, no effect of pH was expected on expressible moisture measurements.

Average total moisture of broken-out liquid egg was 74.6%, providing a solids content (by difference) of 25.4%, within the range of commercial specifications for liquid whole egg (Stadelman et al. 1988; Cotterill, 1990). The main effect on moisture content for the precooked products was the percent ingredient added with near step-wise decrease from 74% moisture to about 71-72% at the highest added ingredient level.

For cooked egg products, loss of moisture during a freeze-thaw cycle (syneresis) occurs as a result of ice crystal damage to the gel structure that characterizes the product. Net syneresis calculated on the basis of moisture differences between precooked, refrigerated product and precooked, frozen and thawed products was found to be 0.81-1.94%. This data could not be compared with other freeze-thaw data previously reported because some moisture gain occurred in the water cooking procedure used in this study.

Controlling free liquid exudation from precooked and frozen products on thawing addresses only a product surface problem because internally water in the egg gel structure remains in enlarged pores or channels created by ice crystal formation during freezing (Bengtsson, 1967). Expressible moisture from application of centrifugal force was used to determine effectiveness of each added ingredient to restrict free water and maintain it within the hydrated gel structure. Comparisons of cornstarch and modified cornstarch effects on expressible moisture are shown in Table 2. There is an overall decrease ($P<0.05$) due to concentration increase for cornstarch whereas higher loss of moisture occurred when modified cornstarch was used.

When the protein ingredients are compared for concentration (1-5%) effects on expressible moisture, egg albumen showed no effect. Soy protein and sodium caseinate decreased ($P<0.05$) expressible moisture as concentration of each ingredient increased (Table 3). Freezing and thawing of the precooked egg products increased expressible moisture for each of the ingredients. However, using the control, non-frozen precooked whole egg product as the “target” for moisture retention (29.3% expressible moisture), the only frozen-thawed products that had equivalent or greater moisture retention were those containing ≥3% sodium caseinate (range of 30.8-21.4% expressible moisture).

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Of headspace volatiles, hexanal and pentanal were used as indicators of oxidative degradation products. Cooking, freezing and reheating will generally increase the rate of lipid oxidation in food products. For products containing added egg albumen, no effect ($P>0.05$) due to its addition was found in the concentrations (in peak areas) of pentanal and hexanal, whereas the concentrations increased ($P<0.05$) with addition of soy protein and decreased ($P<0.05$) with addition of sodium caseinate (Table 4). Based on these specific volatiles, the data indicates that sodium caseinate will inhibit oxidation in cooked whole egg exposed to the freeze-thaw-reheat process and soy protein likely increases the oxidation rate.

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Table 1. pH of whole egg at various stages of product preparation.

Whole Egg Sample	pH	SEM	Range in pH
Fresh broken-out whole egg	7.72	0.06	7.65-7.82
Fresh with pH adjustment	7.02	0.02	6.99-7.06
With added ingredients (over all)	7.86	0.02	6.63-8.09
Freshly cooked (over all ingredients)	8.03	0.01	7.76-8.24

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Table 2. Effect of concentration of cornstarch and modified cornstarch on expressible moisture content of precooked whole egg product.

Concentration (%)	Expressible Moisture (%)	
	Corn Starch	Modified Corn Starch
0.0	48.7 a	47.4 a
1.5	46.4 ab	49.3 a
3.0	44.6 bc	49.8 ab
4.5	43.7 bc	53.5 b
6.0	43.0 c	61.4 c

[a-c] Column means not having a common letter differ (P<0.05).

Table 3. Effect of concentration on expressible moisture for protein ingredients used in precooked non-frozen and precooked frozen-thawed whole egg products.

Ingredient	Percent	Expressible Moisture (%)	
		Non-frozen	Frozen-Thawed
Control	0	29.3	48.9
Egg Albumen	1	29.2 a	48.7 a
	2	30.2 a	46.1 a
	3	28.5 a	45.1 a
	4	28.4 a	44.8 a
	5	29.3 a	43.8 a
Soy Protein	1	27.3 a	45.7 a
	2	27.1 ab	44.3 a
	3	22.6 bc	41.9 ab
	4	21.8 c	41.9 ab
	5	20.8 c	37.0 b
Na Caseinate	1	21.8 a	37.9 a
	2	18.9 ab	37.4 a
	3	16.1 abc	30.8 b
	4	13.4 bc	25.1 c
	5	12.0 c	21.4 c

[a-c] Column means within an ingredient not having a common letter differ (P<0.05).

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Table 4. Effect of soy protein and sodium caseinate on headspace volatiles pentanal and hexanal (peak areas) in precooked, frozen-thawed and reheated whole egg.

Percent	Soy Protein		Na Caseinate	
Ingredient	Pentanal	Hexanal	Pentanal	Hexanal
0	1.35 cd	4.27 d	2.01 a	5.12 a
1	1.23 d	4.61 d	1.46 b	5.09 a
2	1.52 bc	6.43 c	1.14 c	3.84 ab
3	1.59 b	7.43 b	1.07 c	3.50 b
4	1.70 ab	8.13 b	0.99 c	3.12 b
5	1.83 a	9.43 a	0.99 c	3.08 b
(SEM)	(0.07)	(0.30)	(0.08)	(0.59)

[a-c] Column means not having a common letter differ ($P<0.05$).

CONTENTS

DEVELOPMENT OF A CREMA CATALANA-LIKE CUSTARD BY HIGH PRESSURE PROCESSING

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Keywords : custard, rheology, yolk, high pressure

Abstract

The aim of the present study was to design a Catalan custard formulation by high pressure processing and to compare the functional and textural properties with those of the product obtained by heat processing. The use of pressure with different liquid egg yolk proportion and different pH was evaluated and compared with conventional heat processing. All samples formed stable gels under pressure. The pH had a marked effect in all the results. Pressurized-samples had a thixotropic and shear thinning behavior. The hysterisis area was greater for pressure-induced gels than for those obtained by heating. Differences in both the value of shear stress and the analysis of yield point were observed. Gels obtained by heat-treatment presented higher lightness than thoseobtained by pressure-treatment.

Résumé

L'objectif de la présente étude a été de concevoir une formule de crème catalane par traitement haute pression et de comparer ses propriétés fonctionnelles et sa texture à une crème obtenue par traitement thermique. L' application du traitement par pression à des crèmes formulées avec différentes proportions de jaune d'œuf liquide et différents pH a été évaluée et comparée aux mêmes crèmes traitées thermiquement. L'aire de histérisis était plus grand pour les gels obtenus par le traitement par pression que pour ceux obtenues par le traitement thermique. Des différences dans la valeur de la force de cisaillement et dans l'analyse du point de flux ont été observées. Les gels obtenus par traitement thermique ont présenté une plus grande clarté que ceux obtenus par traitement par pression.

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Introduction

The functional properties and nutritional value of the yolk are exploited in many traditional recipes and in novel egg products. Catalan custard, a quite popular dessert in Spain, is a mix of milk, egg yolk, starch and sugar, subjected to heat. Novel product design and application of novel technology are needed for food industries to be competitive in the market. In recent years, application of high pressure processing to foods has been a growing interest as alternative to heat processing (Knorr, 1995). By this way, food products with less modifications, the same or longer shelf-life and new textural properties can be obtained (Cheftel, 1992). High pressure offers many opportunities because it influences functionality, structure and texture (Iametti et al., 1999; Ponce, 2000). Maintaining the native color and flavor of foods high pressure avoids the use of additives (Farr, 1990).

Material and Methods

Raw material

Skimmed milk powder, sugar and fresh eggs were purchased in a local supermarket. The egg yolk and chalazas were separated manually. Three formulations were prepared with constant skimmed milk powder (6%), sugar (1.5%), ι-carrageenan (0.6% - CP Kelco, Terrasa, Spain), 20, 25 and 30 % egg yolk and 71.9, 66.9 and 61.9% of water, respectively. All the ingredients were stirred using a Ultraturrax mixer (Heidolph, Germany). Mixes were divided in two batches and the pH was adjusted to 5.5 and 7.0 with NaOH (0.5 N) and/or HCl (3 N).

Heat and pressure treatments

Samples were put inside a non-edible cellulose casing (Nojax Viskase, Boagnolet, France) of 2.2 cm diameter. The thermal processing was performed in a water bath at 75 ºC for 20 min. Pressure processing was performed in a discontinuous isostatic press (Stansted Fluid Power; LTD, UK) at 500 and 600 MPa for 20 min at 40 ºC. Both treatments, heat and pressure, were performed three times. All samples were kept at 4ºC until analysis were performed.

Rheological study

Pressurized and heated samples were maintained for 2 hours at room temperature, then they were carefully taken out of the cellulose casing and were cut into disks of 0.5 cm in height. Rheological measurements were made with the Rheometer RSI (Thermo Haake, Germany) equipped with PP35 Serrated (two parallel plate) measuring head, the distance between plates were 1.00 mm. The thixotropic and controlled deformation tests were run. The hysteresis area was measured using an increasing shear rate from

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0.00 to 50 s^{-1} in 60 s to define the upward ramp; after an intermediate time of 30 s, the downward ramp was measured diminishing the shear rate from 50 to 0.00 s^{-1} in 60 s. The controlled deformation was evaluated with a minimum/maximum test, which obtained a gradual increase of deformation through the time.

Color

Samples were analyzed for triplicate with a HunterLab spectrocolorimeter (Hunter Associates laboratory, Reston, V). Measurements were made with reference to illuminant F_{cw} (cool white Fluorescent) and 10º standard observer. The samples were put in a quartz small boat, and L, a and b values were measured.

Statistical analysis

Analysis of variance was performed using the General Linear Models procedure of SAS (SAS Institute, Cary, NC) LSMEANS test was used to compare among sample means. Significance level was set at $p< 0.05$.

Results and discussion

The composition and initial pH of mixes are showed in table 1. The results of the thixotropic test are showed in table 2. The results showed that all samples analyzed had a shear thinning behavior. In addition, that the proportion of yolk, pH and treatment influenced (in many cases) significantly the hysterisis area. Samples at pH 5.5 and 30% yolk treated at 600 MPA showed a thixotropic behavior statistically similar to those obtained by heating, both samples had a minor hysterisis area; therefore, they had greater elastic behavior.

Samples with pH 5.5 and treated at 500 MPa, presented a greater hysterisis area than the samples treated at 600 MPa and those obtained by heat processing. The value of hysterisis area represented a greater value of the deformation. This means that the reorganization for Brownian movements of the links of the structural elements that form these systems was slow. Then, the samples need more time to rebuilt its structure or need a longer relaxation time, according to Rosenthal (2001).

The controlled deformation test showed that there is a time dependence. Samples with pH 5.5 and 30% of yolk treated at 500 MPa showed the higher shear stress deformation value. A great influence of pH in the plastic or elastic characteristics was observed. Results about the shear stress needed for the structure deformation and to reach the yield point are showed in table 3. The results indicated that the gelation mechanism was different between gels obtained by pressure and by heat. Pressure denaturation of protein was caused as a result of the decrease in the volume of the protein solution (Okamoto et al., 1990).

Results for color analyses are in table 4. For all mixes, both pressure and heat treatment induced changes in all parameters. Samples at pH 7.0 presented lower lightness than those at pH 5.5. Mixes treated with pressure had lower lightness than mixes treated with heat. The lightness of non-treated mixes was inversely correlated to the yolk proportion. The a and b values were greater for 30% yolk and lower at pH 5.5.

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Conclusions

It is possible to obtain a Catalan custard by high pressure processing with higher proportion of egg yolk and no sugar. The pH of the mix is a main factor in the reological properties, neutral pH gives the more similar aspect to the commercial custards. Samples with 30% of yolk or pH 5.5 were very hard. Samples treated by pressure at 600 MPa and by heat have very similar thixotropic characteristics, with an elastic behavior. Very few differences are observed between treatments in color parameters, although all samples differ from the original mixes.

TABLE 1. **Composition and pH**

Determination	Formula 1	Formula 2	Formula 3
Moisture %	83.13 +/- 0.28	79.17 +/- 0.57	78.33 +/- 0.10
Protein %	5.2 +/- 0.23	5..95 +/- 0.14	6.48 +/- 0.15
Fat %	6.05 +/- 0.07	6.30 +/- 0.03	6..50 +/- 0.08
Ash %	0.80+/-0.12	0.87+/-	1.05+/-0.03
Carbohydrates*	3.54	5.14	7.64
pH	6.74	6.71	6.63

*% obtained by difference

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TABLE 2. Hysterisis area value

Formulations	pH	Treatment	Hysteresis (Pa/s)
Formulation 1 20% yolk	5.5	600 MPa	5608.8 [c] +/- 1956.8
		500 MPa	7979.0 [c] +/- 1956.8
		Heat	4270.8[d] +/- 1694.6
	7.0	600 MPa	2676.8 [d] +/- 19568
		500 MPa	2215.0 [d] +/- 1770.0
		Heat	2436.7 [d] +/- 1770.0
Formulation 2 25% yolk	5.5	600 MPa	7512.4 [c] +/- 2218.8
		500 MPa	15427 [b] +/- 1694.6
		Heat	6416.5 [c] +/- 1770.0
	7.0	600 MPa	3153.0 [d] +/- 1956.8
		500 MPa	2941.3 [d] +/- 1770.0
		Heat	3637.7 [d] +/-1694.6
Formulation 3 30% yolk	5.5	600 MPa	10642 [c] +/- 1956.8
		500 MPa	32353.3 [a] +/- 1694.6
		Heat	9118.3 [c] +/- 1694.6
	7.0	600 MPa	3056.0 [d] +/- 1956.8
		500 MPa	2390.8 [d] +/- 1694.6
		Heat	3609 [d] +/- 1770.0

*Means within a variable with no common superscript differ significantly ($p < 0.05$)

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TABLE 3. Maximum stress deformation value

Formulations	pH	Treatment	Max stress(Pa)
Formulation 1 20% yolk	5.5	600 MPa	440.3 [d] +/- 76.0
		500 MPa	682.0 [c] +/- 72.0
		Heat	341.9 [d] +/- 65.8
	7.0	600 MPa	192.8 [e] +/- 76.0
		500 MPa	173.8 [e] +/- 68.7
		Heat	193.8 [e] +/- 68.7
Formulation 2 25% yolk	5.5	600 MPa	518.2 [c] +/- 86.2
		500 MPa	993.3 [b] +/- 65.8
		Heat	489.4 [c] +/- 68.7
	7.0	600 MPa	245.6 [d] +/- 76.0
		500 MPa	216.8 [e] +/- 68.7
		Heat	249.4 [d] +/- 65.8
Formulation 3 30% yolk	5.5	600 MPa	754.6 [c] +/- 76.0
		500 MPa	1639.8 [a] +/- 65.8
		Heat	628.8 [c] +/- 65.8
	7.0	600 MPa	189 [e] +/- 76.0
		500 MPa	160.6 [e] +/- 65.8
		Heat	228.3 [e] +/- 68.7

*Means within a variable with no common superscript differ significantly ($p < 0.05$)

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TABLE 4. Color analysis values

Formulation	pH	Treatment	L	a	b
Formulation 1 20% yolk	5.5	600 MPa	80.0 ^{b} +/- 0.4	4.0 ^{b} +/- 0.3	19.4 ^{c} +/-0.3
		500 MPa	79.5 ^{c} +/- 0.4	3.7 ^{b} +/- 0.3	19.2 ^{c} +/- 0.3
		Heat	82.5 ^{a} +/- 05	3.6 ^{b} +/- 0.3	18.9 ^{c} +/- 0.3
		N.T.	80.0 ^{b} +/- 0.5	2.7 ^{c} +/- 0.5	18.7 ^{c} +/-0.5
	7.0	600 MPa	76.3 ^{e} +/- 0.4	3.8 ^{b} +/- 0.3	18.4 ^{c}+/- 0.3
		500 MPa	76.6 ^{e} +/- 0.4	4.0 ^{b} +/- 0.3	19.9 ^{b}+/- 0.3
		Heat	78.7 ^{c} +/- 0.4	4.3 ^{a} +/- 0.3	20.6 ^{b} +/- 0.3
		N.T	81.4 ^{a} +/- 0.4	3.5 ^{b} +/- 0.3	18.0 ^{d} +/- 0.3
Formulation 2 25% yolk	5.5	600 MPa	80.4 ^{b} +/-0.4	4.1 ^{b} +/- 0.3	18.9 ^{c}+/- 0.3
		500 MPa	80.0 ^{b} +/-0.4	3.7 ^{b} +/- 0.3	19.0 $^{c+}$/- 0.3
		Heat	82.0 ^{a} +/- 0.4	4.1 ^{b} +/- 0.3	19.7 ^{c}+/- 0.3
		N.T.	79.3 ^{c} +/- 0.5	3.8 ^{b} +/- 0.5	20.4 ^{b} +/- 0.5
	7.0	600 MPa	76.3 ^{e} +/- 0.4	4.7 ^{a} +/- 0.5	20.3 ^{b}+/- 0.3
		500 MPa	74.9 ^{f} +/- 0.4	4.8 ^{a} +/- 0.3	20.7 ^{b} +/- 0.3
		Heat	78.4 ^{d}+/- 0.4	5.4 ^{a} +/- 0.3	21.8 ^{a}+/- 0.3
		N.T	80.6 ^{b} +/-0.4	4.2 ^{b} +/- 0.3	19.2 ^{c} +/- 0.3
Formulation 30% yolk	5.5	600 MPa	79.7 ^{b}+/- 0.4	4.1 ^{b} +/- 0.3	19.1 ^{c} +/- 0.3
		500 MPa	78.1 ^{d} +/-0.4	4.2 ^{b} +/- 0.3	20.0 ^{b}+/- 0.3
		Heat	79.6 ^{b} +/-0.4	4.7 ^{a} +/- 0.3	21.7 ^{a}+/- 0.3
		N.T	77.9 ^{d} +/- 0.4	5.4 ^{a} +/- 0.3	21.9 ^{a} +/- 0.3
	7.0	600 MPa	73.5 ^{e} +/- 0.4	5.2 ^{a} +/-0.3	21.1 ^{a}+/- 0.3
		500 MPa	73.3^{e} +/- 0.4	5.5 ^{a} +/- 0.3	21.6 ^{a} +/- 0.3
		Heat	77.6 ^{d}+/- 0.4	5.2 ^{a}+/- 0.3	21.9 ^{a} +/- 0.3
		N.T	77.0 ^{e} +/- 0.4	5.1 ^{a}+ /-0.3	21.4 ^{a} +/- 0.3

*Means within a variable with no common superscript differ significantly ($p < 0.05$)

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Session 13
Biological properties of egg and egg products

PROPERTIES AND APPLICATIONS OF PROTEINS EXTRACTED FROM HEN EGG WHITE

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Keywords: Egg white, proteins, lysozyme, application

Abstract

Egg white contains numerous proteins having an anti-microbial function that are part of a system of defence designed to protect the chick at the embryonic stage. Lysozyme is the most important one, but other proteins like avidin and ovotransferrin play a key role in this defence system as well. Because of their antimicrobial properties, these three proteins can be used as food protectants (lysozyme in cheese and wine) and in various pharmaceutical applications (lysozyme, avidin and ovotransferrin).

Résumé

Le blanc d'œuf contient plusieurs protéines ayant un pouvoir anti-microbien et qui font partie d'un système de défense destiné à protéger le poussin au stade embryonnaire. Le lysozyme est la plus importante d'entre elles, mais d'autres protéines comme l'avidine et l'ovotransferrine jouent également un rôle important dans ce système de défense. En raison de leur pouvoir anti-microbien, ces trois protéines peuvent être utilisées pour protéger des denrées alimentaires (lysozyme dans le fromage ou le vin) ou dans différentes applications pharmaceutiques (lysozyme, avidine et ovotransferrine).

Occurrence, structure and properties of the main egg proteins

Occurrence

As the vessel that is designed to house the growing offspring of a bird or reptile, it is not surprising that an egg contains numerous proteins having an antimicrobial

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function. By far the most widely studied of these is the small, but significant, lytic enzyme known as lysozyme.

Lysozyme was first identified in 1921 in human nasal secretion by the man who would later discover penicillin, Alexander Fleming. As with many brilliant discoveries, Fleming's skills in looking beyond the obvious led to his discovery of the antimicrobial properties of these two compounds. Lysozyme has since been isolated in human tears, saliva and mother's milk, as well as viruses, bacteria, phage, plants, insects, birds, reptiles and other mammalian fluids. Commercially, the most readily available source of lysozyme has been the egg white of the domestic chicken (Gallus gallus). Lysozyme is likely the most scientifically studied protein of all. It was the first protein to have its structure determined by X-ray crystallography in the 1960's. It is used as a molecular weight marker in analytical procedures, such as electrophoresis and chromatography and is used in many labs to produce spheroplasts – micro-organisms whose cell wall is removed to expose the inner membrane.

Lysozyme is not, however, the only protein of interest in eggs. It forms only one part of a system of defence that is designed to protect the chick at the embryonic stage.

Table 1 lists a number of proteins found in avian egg white. By far, the majority of research to date has focused on the proteins of egg white rather than the egg yolk. This may be due to the relative ease with which many of the proteins in egg white can be isolated by direct application to ion-exchange media.

Most yolk proteins, on the other hand, tend to be less water soluble, and because of their close association with lipids, they are somewhat less easily extracted and purified on a large scale. Nevertheless, there are antimicrobial proteins present in the yolk, including immunoglobulins (IgY - the chicken equivalent of IgG) and trace amounts of a biotin binding protein. It is likely that any antimicrobials in the yolk, however, are meant primarily to provide the developing chick with passive protection, as is the case with the immunoglobulins. At present, IgY is the most studied yolk protein, and appears to hold the greatest potential for use as a food or feed additive.

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Protein	Percent of Solids	Function
Ovalbumin	**54**	Unknown
Ovotransferrin	**12**	Binds multi-valent cations, particularly iron
Ovomucoid	**11**	Inhibits trypsin and other proteases, antimicrobial properties
Ovoinhibitor	**15**	Inhibits trypsin, chymotrypsin and other proteases
Ovomucin	**3.5**	Increases viscosity of egg white preventing bacterial movement
Lysozyme	**3.4**	Lyses peptidoglycan layer of some Gram- positive bacteria
Ovoflavoprotein	**0.8**	Binds riboflavin (vitamin B2)
Ovomacroglobulin	**0.5**	Protease inhibitor
Ficin Inhibitor (Cystatin)	**0.05**	Inhibits cysteine proteases
Avidin	**0.05**	Binds avidin, making it unavailable to micro-organisms

Table 1. Some of the major proteins in egg white and their antimicrobial functions

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The three egg proteins that appear at present to hold the most potential, as natural antimicrobials in food and pharmaceutical applications are lysozyme, avidin, and ovotransferrin.

Structure

It is now recognized that lysozymes from diverse sources fall into several structural classes, with the three most common being type c (chicken), type g (goose) and type v (viral). Chicken lysozyme is composed of 129 amino acid residues with a molecular weight of approximately 14,400 daltons.

Lysozyme is used extensively as a model enzyme, partly because it contains all of the twenty common amino acids. It contains four disulphide bridges, and has portions that are helical and antiparallel β-sheet. An α-helix links two domains of the molecule, one mainly β-sheet in structure, and the other primarily α-helical. As with many proteins, the hydrophobic groups are mainly oriented inward, with most of the hydrophilic residues on the exterior of the molecule.

It is believed that the enzymatic action of the molecule is dependent on its ability to change the relative position of its two domains by hinge bending. Essentially, the small α-helical connection, or hinge, between the domains can bend sufficiently to cause large conformational changes in the molecule, permitting the enzyme to gain access to its substrate.

Avidin is a glycoprotein composed of four identical subunits, each with 128 amino acid residues. The molecular weight of the entire molecule is around 67,000 daltons. A disulphide bridge links residues four and eighty-three and there are four tryptophan residues per subunit.

Ovotransferrin, also known as conalbumin, is a glycoprotein with a molecular weight of 78,000 daltons. Like lysozyme, it contains two lobes connected by an α-helix. In the case of ovotransferrin, however, each lobe is homologous, and can bind an Fe3+ ion. The iron-binding site in each lobe is situated between two sub-domains. The presence of bicarbonate ion enhances the binding of iron to the molecule.

Properties

Lysozyme has an extremely high isoelectric point (>10) and consequently is highly cationic at neutral or acidic pH. In solutions with pH of 3 or 4, lysozyme is relatively stable and can withstand near boiling temperatures for a few minutes. As solution pH increases, however, lysozyme's stability decreases. In the pH 5.5 to 6.5 range (which includes many dairy products), lysozyme is stable up to about 65°C. Beyond this point, denaturation accelerates rapidly with temperature. Thus many pre-cooked food products, which typically achieve temperatures in the 69 to 75°C range, encounter a temperature that may partially or completely denature the enzyme, depending on the pH.

Avidin is unique in that its complex with biotin results in an association coefficient (Ka) of about 10^{15}, the strongest such biological association known that does not

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involve covalent bonding. Avidin is inactivated at 85°C, but the avidin-biotin complex can withstand temperatures greater than 100°C for a brief period.

Ovotransferrin is somewhat less cationic, with an isoelectric point of 6.1. Each molecule is capable of binding two atoms of iron. Ovotransferrin will also bind aluminum and copper ions, with the order of strongest to weakest binding being $Fe^{3+} > Al^{3+} > Ca^{2+}$.

Mode of Action

The primary mechanism by which lysozyme lyses micro-organisms is by enzymatically cleaving the bonds between the C4 of N-acetylglucosamine and the C1 of N-acetylmuramic acid, the two repeating units of the peptidoglycan layer. Against organisms with a relatively accessible peptidoglycan layer (Gram-positive bacteria), lysozyme is fairly active. Against organisms where this layer is not as accessible (for example, Gram-negative bacteria), the enzyme is not able to access its substrate and shows little or no antimicrobial effect. Compounds which help to destabilize the outer membrane of gram negative organisms (e.g. E.D.T.A) appear to permit lysozyme to act on some of these otherwise unassailable targets.

The antimicrobial properties of lysozyme are not only limited to its enzymatic action. It is also believed that, by adhering to the exterior surface of some micro-organisms (especially fungi), it can interfere with cell function and hinder growth and replication. It can also form complexes by acting as the positively charged binding agent between the negatively charged outer surface of micro-organisms.

Avidin's antimicrobial effect is attributed to its ability to strongly bind with biotin. By depriving micro-organisms of this essential nutrient, it can have a bacteriostatic effect on biotin-reliant organisms.

Although some researchers have attributed the antimicrobial effect of ovotransferrin to its ability to bind iron, and thereby deprive iron-dependent organisms of an essential element, recent studies have shown that the bactericidal effect is relatively independent of the degree of iron saturation of the molecule. It is now believed that a peptide sequence embedded within the primary structure may in fact cause direct disruption of cell surfaces. This is based on knowledge of similarities in structure between ovotransferrin and lactoferrin, a milk transferrin containing a bactericidal peptide sequence known as lactoferricin. Recent research has identified a ninety two-residue sequence in the N-lobe portion of ovotransferrin that displays antimicrobial effect against Staphylococcus aureus and Escherichia coli K-12. Three disulphide bridges within this sequence contribute to a tertiary structure that is needed for the antimicrobial action.

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Importance in food and pharmaceutical applications

Lysozyme has been used in pharmaceutical and food applications for many years, due to its lytic activity on the cell wall of gram-positive micro-organisms. These bacteria are responsible for infections of the human body and for the spoilage of various foods.

Pharmaceutical Applications of Lysozyme

Hen egg white lysozyme is used in "over-the-counter" drugs, in order to increase the natural defenses of the body against bacterial infections. The pharmaceutical use of lysozyme encompasses applications such as otorhino-laryngology (lozenges for the treatment of sore throats and of canker sores), and in ophthalmology (eye drops and solutions for the decontamination of contact lenses).

Lysozyme is also added to infant formulae in order to make them more closely resemble human milk (cow's milk contains very low levels of lysozyme).

A recent publication has renewed interest in the antiviral properties of lysozyme by demonstrating anti-HIV activity of both human and chicken lysozymes in vitro. It is possible that these findings may lead to new means of treatment of this infection.

Food Applications of Lysozyme

Much research has been done on the use of lysozyme as a preservative in food products, particularly in the Far East and Japan. Several applications have been developed and patented, including the treatment of fresh fruits, vegetables, seafood, meat, tofu, sake and wine.

However, the most important food application of lysozyme is the prevention of a problem known as "butyric late blowing", which occurs during the ripening of certain European-type cheeses. This problem is due to the contamination of milk by a naturally occurring, spore-forming bacterium, called *Clostridium tyrobutyricum*. The origin of this contamination lies in the widespread use of silage as a feed. The spores of *Clostridium tyrobutyricum* are present in the soil and incorporated, together with some particles of soil, into the silage when it is harvested. The spores will then proliferate in the silage if an intense acidification does not take place rapidly. When the cows are fed with the contaminated silage, the spores are excreted in the manure and, if the milking is not carried out under very strict hygienic conditions, the spores can subsequently contaminate the milk. It has been demonstrated that a very small amount of manure (less than one gram) is enough to contaminate a tank containing several thousand gallons of milk.

If cheese is made with milk contaminated with spores of *Clostridium tyrobutyricum*, the majority of the spores are retained in the curd. Here, the conditions (absence of oxygen and presence of large amounts of lactates) are favorable for germination and for the development of vegetative forms during the ripening of the cheese. Lactic acid can be metabolized as the primary source of by *Clostridium tyrobutyricum* to produce butyric acid and a blend of two gases: hydrogen and carbon dioxide. The

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accumulation of butyric acid is responsible for organoleptic defects in the cheese, due to the characteristic off-flavour caused by this short chain fatty acid. The production of large volumes of hydrogen (totally insoluble in the water phase of the cheese curd) and of carbon dioxide (partly soluble) leads to an increase in the internal pressure of the cheese and, subsequently, to the formation of slits and cracks in the cheese during the ripening process. This has a dramatic and detrimental impact on the quality of the cheese and, consequently, on its commercial value. Cheese with a late blowing problem usually has to be downgraded or, in severe cases, cannot be sold at all.

Research begun in the late 1960's and 1970's, followed by cheese trials carried out in Europe in the early 1980's, has demonstrated the efficacy of lysozyme to prevent late blowing in different types of cheese. The principle of lysozyme action is based on its capacity to be retained in the cheese curd, through electrostatic attraction with the casein, and on the stability of its enzymatic activity throughout the ripening process. Lysozyme is active on the vegetative cells of *Clostridium tyrobutyricum*, which appear during the ripening process. The usage level is usually 25 ppm in the cheese milk. Most of the lactic cultures used in the production of cheese, although Gram-positive bacteria, are not sensitive to the lytic action of lysozyme at this dosage.

Lysozyme has been approved by the European Union, and has now been used with success for more than fifteen years in several European countries (e.g. France, Italy, Spain, Portugal, Germany, Denmark, The Netherlands, etc.). Its use has also been successful in different types of cheeses, like the hard cheeses (Parmesan, Swiss), the semi-hard cheeses (Gouda, Manchego), and the soft cheeses (Brie). Lysozyme has recently received GRAS (Generally Recognized as Safe) status from the Food and Drug Administration of the United States, and is raising a lot of interest in North America for its application in specialty cheeses.

A new food application has been recently developed, which consists in the total or partial substitution of sulfites by lysozyme in wine. Due to its inhibitory power on lactic acid bacteria, lysozyme can effectively control the development of the malo-lactic fermentation in white and red wines. Several experiments carried out in Europe have demonstrated that the use of lysozyme allows the elimination, or at least a substantial reduction, of the amount of sulfites normally added to the wine for the control of the malo-lactic fermentation. Lysozyme leads, therefore, to an improvement of the organoleptic properties of the wine (color and taste) and a better tolerance by the consumers. The use of lysozyme in the wine has been allowed in the European Union since October 2001.

Because lysozyme is mainly effective against Gram-positive bacteria, recent research has also focused on synergistic combinations of lysozyme and other antimicrobial compounds such as nisin. These combinations often target the Gram-negative food spoilage bacteria against which lysozyme is ineffective alone.

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Applications of Avidin

Avidin, in conjunction with biotin, is used in a number of diagnostic and analytical applications, including biotinylated probes for a number of quantitative detection methods, affinity chromatography columns, immunoassays, immunohistochemistry and protein blotting.

Applications of Ovotransferrin

Although patents from the 1970's promoted ovotransferrin as a potential inhibitor of mildew in such Asian dishes as noodles, wonton and fried bean curd, it has not been used extensively for its antimicrobial properties in food applications. More recent patents have been applied for using ovotransferrin to treat HIV and to prevent periodontal disease. In Japan, immobilized ovotransferrin has been used to remove iron from drinking water, as well as water for brewing. Also, a company in the Netherlands has recently filed a patent for a nutraceutical drink containing ovotransferrin.

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HEN EGG WHITE LYSOZYME AND OVOTRANSFERRIN : MYSTERY, STRUCTURAL ROLE AND ANTIMICROBIAL FUNCTION

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Keywords: Lysozyme, ovotransferrin, structural motif, defensin-like, antimicrobial peptides

Abstract

Antimicrobial system of hen egg is the one in which enzymes are not yet shown to play role and cells other than the immunologically incompetent ones of the embryo are absent. By contrast with the immune system of animals, which produce antimicrobial polypeptides when needed, the egg albumin efficiently resists micro-organisms over a long period of time in the absence of such professional immune cells. The wide diversity in the nature of micro-organisms imply that albumin, the second defense line of avian eggs, would possess multiple antimicrobial systems. It raise the tantalizing question of whether egg albumin have a complement-like or defensin-like system . We thought that the focus must therefore be on maximizing the value of egg albumin through systematic and knowledge based research for the utilization of egg white proteins in food and drug systems. The egg albumin constituents offer tremendous opportunities. Although egg albumin represents a rich source of proteins with diverse food properties for nutritional, biological, and functional applications, commercial exploitation of these proteins has not been widespread because of the limited research base, a lack of definite physiological function, and inconsistency in interpretating the biological functions because most of them were studied separately without giving account to the possible interconversion of their structures and the complex interactions between the various components that make up the albumin system at physiological temperatures. In this study we attempt to elucidate the structural-antimicrobial relationships of intact and fragmented lysozyme and ovotransferrin, which are relevant to interconversion of their structures under physiological conditions. The data provide new insights into their physiological roles and thus introducing new candidates as novel antimicrobial polypeptides with potential use in the fast growing technology of nutraceuticals.

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Résumé

Le système antimicrobien de l'œuf de poule est l'un de ceux dans lesquels le rôle des enzymes n'a pas été démontré et au sein duquel les cellules immunocompétentes de l'embryon sont absentes. Contrairement aux animaux dont le système immunitaire produit des polypeptides antimicrobiens quand nécessaire, le blanc d'œuf résiste efficacement aux micro-organismes sur de longues périodes malgré l'absence de telles cellules immunitaires. La grande diversité des micro-organismes implique par ailleurs que le blanc d'œuf, qui constitue la deuxième barrière des œufs d'oiseaux, possède de multiples systèmes antimicrobiens. Ce qui soulève la question terriblement alléchante de l'existence dans le blanc d'œuf d'un système « complement-like » ou « defensin-like ». C'est pourquoi nous pensons qu'il faut à présent nous focaliser sur la maximisation de la valeur du blanc d'œuf, via une recherche fondamentale systématique pour l'utilisation des protéines de blanc d'œuf dans les aliments et les médicaments. Les constituants du blanc d'œuf offrent d'énormes perspectives. Bien que le blanc d'œuf représente une source protéique riche avec des propriétés variées pour des applications nutritionnelles, biologiques et fonctionnelles, l'exploitation commerciale de ces protéines n'a pas été développée ; les raisons en sont le déficit en matière de recherche, l'absence de fonction physiologique définie, et les difficultés à interpréter leurs fonctions biologiques parce que la plupart de ces protéines ont été étudiées isolément, sans prendre en compte ni leurs éventuelles modifications structurales, ni les interactions complexes qui peuvent intervenir aux températures physiologiques entre les constituants de ce système complexe qu'est le blanc d'œuf. Nous tentons ici d'élucider les relations entre la structure et les activités antimicrobiennes du lysozyme et de l'ovotransferrine, en relation avec les modifications de leurs structures respectives dans les conditions physiologiques. Ces données apportent un nouvel éclairage sur leurs rôles physiologiques, les présentant ainsi comme de nouveaux polypeptides antimicrobiens potentiellement utilisables dans le vaste secteur en développement des nutraceutiques.

Introduction

Micro-organisms have coevolved with humans, animals, insects, and plants ; most often the relationship is mutually beneficial. In instances where there is competition, both the micro-organisms and the host have had an opportunity to develop defensive strategies. Micro-organisms continually develop resistance to antimicrobial agents. Survival of the host depends on a network of natural defense mechanisms. The natural defense systems generally relies on cellular or humoral immunity. The cellular

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response consists of phagocytosis and encapsulation of micro-organisms by blood cells (phagocytic cells, i.e macrophages and neutrophils). The humoral defenses of vertebrates, invertebrates and plants are essentially based on the synthesis of cationic antimicrobial peptides/polypeptides exhibiting broad activity spectrum (Kazuhara et al., 1990; Lamberty et al., 1999; Lehrer et al., 1991; Viljanen et al., 1988). Antimicrobial peptides are involved in both intracellular (phagocytic cells) and extracellular (serum and epithelial tissues including mucosa) innate defense mechanisms of mammals (Devine and Hancock, 2002). The humoral facet of the insect defense reactions against invading micro-organisms is now clear to be essentially based on a battery of cationic peptides exhibiting antibacterial and antifungal activities. These antimicrobial peptides are produced in the fat body (equivalent of the mammalian liver) and released into the hemolymph (blood) of insects (Lamberty et al., 1999). The most widespread antimicrobial peptides in mammals and insects are defensins (Devine and Hancock, 2002; Lamberty et al., 1999; Lehrer et al., 1991). Defensins and defensin-like antimicrobial peptides comprise astructurally diverse group of low molecular weight which are cationic and amphiphilic with common structural motifs of α-helical, β-sheet and/or cysteine-rich peptides. Human and rabbit defensins are synthesized as 93-95 amino acid preprodefensins with a characteristic 19 residues signal sequence and a 40-45 prosequence. The insect defensin is synthesized as 97 amino acid preprodefensins with a 23 residues signal peptide and a 34 prosequence (Lehrer et al., 1991). Surprisingly, the cytotoxicity of several defensins was found to operate via a mechanism that is dependent on active metabolism of the target cells (Lehrer et al., 1988).

Up to date, there is absolutely no report on defensin-like peptide found in avian eggs. The occurrence of multiple conformational states of proteins in the egg albumin is expected to have important consequences for interpretation of their biological functions (Ibrahim, 1997a). The solids of egg albumin consist mainly of proteins (11 %). During incubation of an egg, diversified inert protein structures interact in a certain fashion via a chain of stages to produce the living chick. Apparently these stages involve several biological interconversion of the constituent proteins (Burley and Vadehra, 1989; Vadehra and Nath, 1973). The roles of this set of proteins in egg white are believed to interfere with the invading micro-organisms from reaching the yolk (nutrients store) and embryo. A concept referred to as the natural antimicrobial cocktails (Banks et al., 1986). Of different proteins found in the egg albumin, most appear to possess antimicrobial properties and physiological functions that interfere with the growth and spread of invading micro-organisms, namely the chemical defense system of avian egg (Tranter and Board, 1982). Lysozyme (Lz) and ovotransferrin (OTf) are believed to constitute the major components of the avian egg's antimicrobial defense system (Tranter and Board, 1982; Tranter and Board, 1984). A dilemma faced food microbiologists, for long time, who wished to explore the role of ovotransferrin and lysozyme in the antimicrobial defense system of avian eggs and their potential

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uses as natural antimicrobial agents. Here, we will summarize the current information and our recent findings on ovotransferrin and lysozyme of the hen's egg albumin, to clarify its structure-antimicrobial relations and the possible role for its existence in the avian eggs, which may allow them to be placed in a clearer structural functional perspective among the natural antimicrobial systems.

Structural Properties of Lysozyme and Ovotransferrin

Lysozyme (muramidase, mucopeptide N-acetylhydrolase) from hen's egg white is a polypeptide of 129 amino acid residues having a molecular weight of 14,300 dalton. It is a basic protein with isoelectric point of 10.7. In the hen's egg white, lysozyme accounts for 3.5% of the total egg white proteins. Lysozyme molecule consists of two domains, linked by a long α-helix, between which lies the active site of the enzyme. The N-terminal domain (residues 40~88) consists of some helices and is mostly antiparallel β-sheet. The second domain is made up of residues 1~39 and 89~129 and its secondary structure is largely α-helical. The two domains of the molecule are separated by a helix-loop-helix motif (Asp 87–Arg 114) which, as we show below, has recently been found to play a key role in its antimicrobial function.

Ovotransferrin (conalbumin) is a glycoprotein consisting of a 686-residues single polypeptide chain with a molecular mass of 78 kDa and isoelectric point of 6.0. In the hen's egg white ovotransferrin accounts for 12% of the total egg white proteins. It has the capacity to bind reversibly two Fe^{3+} ions per molecule concomitantly with two bicarbonate anions. It is a member of three homologous and closely related proteins constituting the transferrin family. The transferrins are a group of iron-binding proteins which are widely distributed in various biological fluids including serum transferrin (iron transport protein; transferrin, siderophilin), lactoferrin (in milk and other secretions), and ovotransferrin (in avian egg white). The transferrins show a remarkable similarity in the amino acid sequence as well as the global folding of the molecules. The avian transferrin gene is expressed both in liver and oviduct, where its protein products are known as serum transferrin and ovotransferrin, respectively. The protein part of avian serum transferrin and ovotransferrin is identical but differ only in the nature of their attached carbohydrate groups. Like the mammalian transferrins, the polypeptide chain of ovotransferrin is folded into two homologous halves (N-terminal lobe, residues 1-332 and C-terminal, residues 342-686 lobe) each with a single iron site. Each lobe is further divided into two domains (N-terminal lobe, N1 and N2 domains; C-terminal lobe, C1 and C2 domains) of about 160 residues each with the iron site between them. The two lobes show marked similarities in their sequences with 37% identical residues. Ovotransferrin contains 15 disulfide bridges; 6

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homologous disulfide bridges in each half of the molecule and 3 extra bridges which occur only in the C-terminal half. When the sequences of the two lobes of hen ovotransferrin are compared with those of human transferrin, all four half-molecules were homologous. The overall structure of hen ovotransferrin (OTf) is similar to human (h-Lf), bovine (b-Lf) lactoferrins, and human or rabbit serum transferrins.

Antimicrobial Activity of Ovotransferrin and Mystery

The antimicrobial activity of ovotransferrin (OTf) is thought to be due to chelation of metal ions, specially iron, thus renders the iron unavailable to microbial growth. Indeed there is no compelling evidence of the starvation strategy in albumin by ovotransferrin since saturation of ovotransferrin with iron does not convert albumin into an optimal medium for the growth of *E. coli* (Banks et al., 1986) nor affected its *in vitro* bactericidal activity to *S. aureus* (Ibrahim et al., 1998). There have been few reports describing the antimicrobial activity of OTf even when complexed with metals such as zinc and iron, implying mechanism other than iron-deprivation. A dilemma faced food microbiologists who wished to explore the role of ovotransferrin in the antimicrobial defense system of avian eggs and its potential uses as natural antimicrobial agent.

Antimicrobial Function of Lysozyme and Mystery

Lysozyme, on the other hand, is an ubiquitous antimicrobial enzyme. The active role played by lysozyme in defense systems in both vertebrates and invertebrates is well recognized. It belongs to a class of enzymes that lyses the cell walls of certain Gram-positive bacteria, by breaking down a component of cell walls. The antibacterial specificity of lysozyme is known to be limited to certain Gram-positive bacteria and is much less effective against Gram-negative ones. The mechanism by which lysozyme kills the sensitive bacteria has long been believed to be the degradation of the glycosidic beta-linkage between N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) of the peptidoglycan layer in the bacterial cell wall.

Although conclusive evidence is lacking, it has long been believed that the antimicrobial action of lysozyme would merely be attributed to its catalytic function on bacterial cell walls. The importance of lysozyme as an active defense molecule has been debated, since in most bacteria the peptidoglycan layer is not directly accessible to this enzyme. Also under physiological conditions only a minority of Gram-positive bacteria are susceptible to lysozyme, because the lytic action of lysozyme does not kill the susceptible bacteria under physiological conditions, osmotically balanced (Brooks et al., 1991). The literature abounds with evidence pertaining to the functional role of lysozyme in various tissues as a defense mechanism suggest that it may primarily be related to structural properties and secondarily to its direct bacteriolytic action (Cisani

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et al., 1989; Laible and Germaine, 1985). Recently, we found that heat-denaturation of lysozyme progressively inactivate the enzyme while greatly promotes its antimicrobial action to Gram-negative bacteria. Surprisingly, denatured lysozyme devoid of enzyme activity exhibited bactericidal activity against Gram-positive bacteria, suggesting action independent of catalytic function (Ibrahim, 1998; Ibrahim et al., 1996a; Ibrahim et al., 1996b; Ibrahim et al., 1997). In another study, we explored that the genetically inactive lysozyme, D52S-Lz (in which the catalytic residue Asp-52 was substituted with Ser) is a potent bactericidal against typical Gram-positive bacteria regardless of its enzyme activity (Ibrahim et al., 2001a). Suggesting antimicrobial action independent of its catalytic function and again like ovotransferrin the antimicrobial action appears to be a structure-dependent mechanism.
Mystery of the antimicrobial behaviour of OTf and Lz suggested a structure-related mechanism of action. Therefore, we intended to clarify the antimicrobial mechanism of these major defense proteins of avian eggs with the objective of verifying the existence of bactericidal peptides within their molecules, such as reported for defensin-like antimicrobial systems within animal and insect kingdoms.

Structural Role and the Discovery of the Antimicrobial Peptides

Ovotransferrin: Structure and Antimicrobial Enigma
Since ovotransferrin is largely free of iron in egg albumin, it has been suggested that its bacteriostatic action in egg white would be caused by depriving the bacteria of iron essential for their growth. Although the studies attributed the antimicrobial effects of ovotransferrin to be bacteriostatic (growth inhibition), the bactericidal (bacterial killing regardless of iron deprivation) action of ovotransferrin against a wide spectrum of micro-organisms has also been suggested. The antibacterial activity of metal complexes of ovotransferrin was studied *in vitro* against different bacterial species and the Zn^{2+}-loaded ovotransferrin appeared to be more active by comparison with the apo-protein and other metal complexes. The effect was specific to the Zn^{2+}-ovotransferrin complex. This antibacterial activity required a direct contact of Zn^{2+}-ovotransferrin with the bacterial surface.
In another study from our laboratory and literatures, the antimicrobial potency of hen egg albumin was boosted at alkaline pH condition (pH 9.5) and by temperature near 40˚C, the physiological temperature of birds. It is now evident beyond doubt that ovotransferrin possesses both the bacterial growth inhibition (static) and the iron withholding-independent (cidal) antimicrobial actions. Exertion of a particular antimicrobial action seems to be environment- and micro-organisms -specific.

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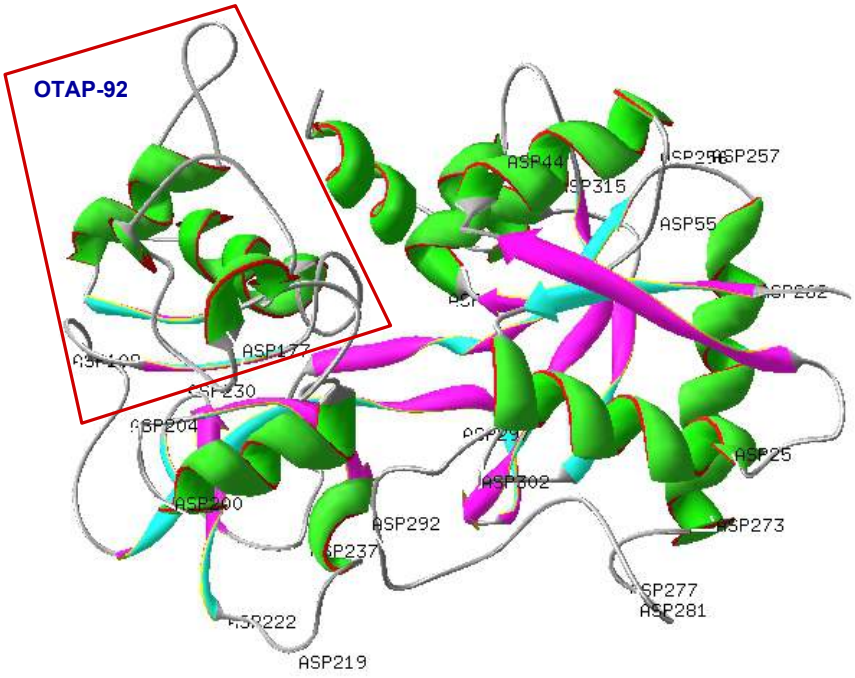

Fig.1. Ribbon representation of the N-terminal half-molecule of ovotransferrin (N-lobe) showing all surface exposed aspartic acid residues are located in loop regions.

A recent work from our laboratory (Ibrahim, 1997b) demonstrated a direct bacterial killing of ovotransferrin against Gram-positive *Staphylococcus aureus* and *Bacillus cereus* and the Gram-negative *E. coli* whether saturated or not with iron. Both of the isolated half molecules of OTf showed remarkable reduction in the CFU of *S. aureus* when freed of iron (Nt-apo and Ct-apo), but the N-terminal lobe exhibited stronger bactericidal activity than the C-terminal lobe. Strikingly, iron-saturation of the half molecules abolished the bactericidal activity of C-terminal lobe (Ct-Fe), while the N-lobe (Nt-Fe) restored its bactericidal effect almost equal in magnitude to the iron-free N-lobe (Nt-apo). Indeed these results confirmed that the bactericidal activity of ovotransferrin is operationally independent of its iron binding properties (nutritional deprivation) and suggested that ovotransferrin possesses antibacterial domain located in the N-terminal lobe.

Structure analysis of Nt-lobe of OTf indicated the presence of a common aspartic acid residue almost at all loop regions (Fig. 1). Therefore, selection of cleavage specificity at Asp residue for this investigation would be of great importance in understanding the

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structure-related antibacterial action of OTf.

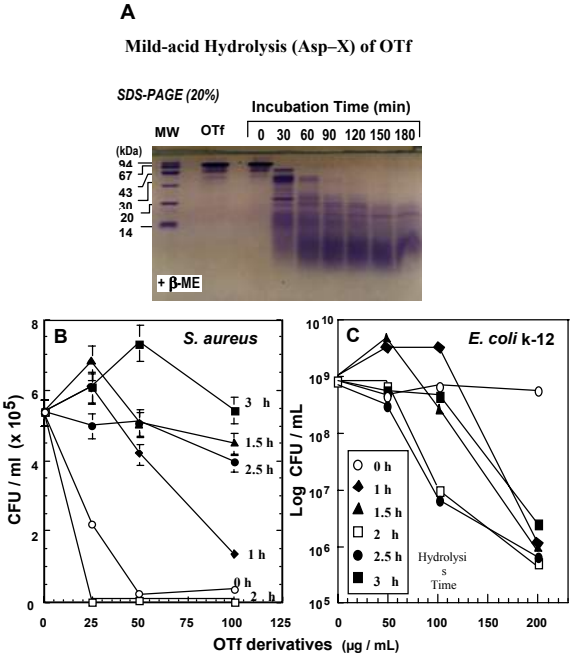

Fig. 2. Cleavage of OTf at Asp-X produces a hydrolysate (A) with potent antimicrobial activity against Gram-positive *Staphylococcus aureus* (B) and Gram-negative *Escherichia coli* K-12 (C).

The isolation of the bactericidal domain of OTf was achieved by utilizing partial-acid proteolysis technique, which produce relatively large peptides via specific cleavage at Asp-X sequence. Hydrolysis at Asp-X sequence produced a strong antimicrobial OTf against Gram-positive and Gram-negative bacteria (Fig. 2). The bactericidal domain of OTf was purified from the active hydrolysate by gel filtration and subsequent reversed-phase HPLC (Ibrahim et al., 1998; Ibrahim et al., 2000). The purified peptide was more bactericidal than the intact OTf or its half-molecules. The isolated peptide showed strong bactericidal activity against both Gram-positive *S. aureus* and Gram-negative *E. coli* strains, where more than six $\log_{10}$ orders of killing against *S. aureus* and more than one $\log_{10}$ order of killing against *E. coli* was observed.

Structural homology of OTf Antimicrobial Peptide with Defensins

Structure analysis of the bactericidal peptide revealed a molecular weight of 9.9 kDa, corresponding to a surface exposed cationic peptide (ca. pI 8.84) consisting of 92

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residues (Leu 109-Asp 200) located at the lip of the iron-binding cleft of the N-lobe of OTf, designated OTAP-92. The OTAP-92 comprised six cysteines engaged in three intrachain disulfide bridges, C115-C197, C160-C174, and C171-C182. Of the three disulfide bridges, two (Cys 160-Cys 174, and Cys 171-Cys 182) formed a cringle bridges, the two disulfides SS-IV and SS-V of OTf. The OTAP-92 domain packed into three helices with a two-stranded β-sheet together with the three disulfide linkages, thus projecting the cringle bridges and a set of basic residues to the exterior of the N-lobe.

Several important structural features were observed in OTAP-92. It is scattered with array of positively charged, which are prerequisites for most antimicrobial peptides. This structural motif of OTAP-92 was found in other transferrins, where all showed the same pattern of cysteines of the two disulfide bridges in the cringle region (Ibrahim et al., 1998). This unique structural motif of OTAP-92, strongly suggests its important role in the biological function of OTf, particularly it is surface exposed and located in the structurally unstable domain (N-lobe).

Multiple alignment showed remarkable sequence similarities between the optimum region of OTAP-92 (Lys 160–Arg 184) and several insect defensins (Fig. 3), who are active in killing both Gram-positive and Gram-negative bacteria (Ibrahim et al., 1998). Although the insect defensins exhibit great structural diversities, the cysteine-rich defensins contain a characteristic cysteine motif analogous to the cringle region of OTAP-92, particularly the cysteine-rich defensins. The cringle region of OTAP-92 also showed sequence similarity, including with the conservative two internal disulfide bridges of these defensins (Ibrahim et al., 1998). Therefore, one apparently universal antibacterial action of OTAP-92 is its relatively high basicity and the striking high degree of conservative cysteines array together with the helix-sheet motif, a characteristic of many native defensins.

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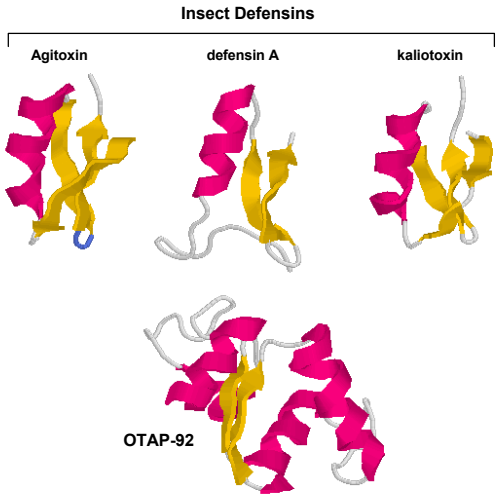

Fig. 3. Ribbon representation comparing the 3D-structures of OTAP-92 and three insect defensins : Agiotoxin, Insect Defensin A and Kaliotoxin.

Outer and inner membrane permeabilization studies provided evidence that OTAP-92 peptides kills the bacteria by crossing the outer membrane via self promoted uptake and causing damage to the inner membrane through channel formation. Therefore, the structural motif of OTf antibacterial peptide (OTAP-92) which shows structural and functional similarities with known bactericidal defensins open the door for its future candidacy as a novel antibiotic peptide in food and pharmaceutical industries as well.

The Novel Antimicrobial Peptide of Lysozyme and Promises

Recently, we showed that the antimicrobial action of chicken egg white lysozyme (cLz) is independent from its enzymatic activity because enzyme lacking catalytic activity still retains its bactericidal properties (Ibrahim et al., 1996a; Ibrahim et al., 2001a). This observation suggests that a specific bactericidal domain may actually be involved in the antimicrobial action of lysozyme. Hence, we digested cLz by a bacterial protease (clostripain) whereas a peptide (residues 98-112) possessing bactericidal activity has been identified (Pellegrini et al., 1997). Of particular interest is that the peptide is a part of a helix-loop-helix (HLH) motif (residues 88-114 of cLz and 87-115 of human lysozyme, hLz) located at the upper lip of the active site cleft of the molecule (Fig. 4).

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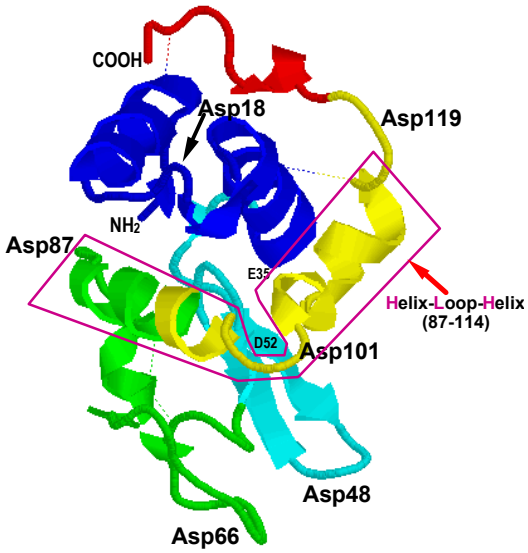

Fig. 4. Ribbon representation of egg white lysozyme showing all aspartic acid residues, except the catalytic residue D52, are located in loop regions. The HLH domain (87-115 sequence) is shown (boxed region).

The helical hairpin (HLH motif) are known structures commonly found in bactericidal and cytolytic pore-forming proteins. For example, cecropin A and B1, major antimicrobial peptides in the immune response of silk moths, consist of two α-helices flanked by a loop maintained by Gly and Pro residues. Colicin E1, a bactericidal protein with the ability to form an ion channel in the *E. coli* cytoplasmic membrane, possesses a C-terminal α-helical hairpin which mediates insertion and subsequent channel assembly into the membrane. Like with ovotransferrin, structure inspection of lysozyme showed that six out of seven aspartic acid residues are located at loop regions whereas the HLH domain is flanked by two Asp residues (Fig. 4).

In order to investigate the structure-microbicidal relationship of lysozyme and its mechanism of action, the full HLH domains of both the chicken and human lysozymes and their secondary structure derivatives were synthesized and tested for antimicrobial activity against several microorganisms. The synthetic peptides were also evaluated for their ability to interact with the membrane of *E. coli*. The antimicrobial domains of the chicken and human lysozymes are helical hairpins whose structures are corresponding to the sequences DITASVNCAKKIVSDGNGMNAWVAWRNR (cP_{87-114} of the chicken lysozyme) and DNIADAVACAKRVVRD PQGIRAWVAWRNR (hP_{87-115} of the human lysozyme). Peptides with the sequences P_{87-114} and P_{87-115} were synthesized and proofed in *vitro* against several Gram-positive and Gram-negative

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bacteria. Both HLH peptides exerted a wide spectrum of bactericidal activity against Gram-negative and Gram-positive bacteria. The HLH peptides were moreover able to inhibit the growth of the fungus *C. albicans*. In order to investigate the importance of the structural element of HLH for bactericidal activity, the peptides DITASVNCAKKIVS ($cP_{87-100,}$ helix1), DGNGMN ($cP_{101-106,}$ loop) and AWVAWRNR ($cP_{107-114}$, helix 2) derived from the chicken lysozyme HLH motif and the peptides DNIADAVACAKRVVR (hP_{87-101}, helix 1) DPQGI ($hP_{102-106,}$ loop), RAWVAWRNR ($hP_{107-115}$ helix2) from the human lysozyme were synthesized and their bactericidal properties investigated. The most bactericidal peptides were $cP_{107-114}$ and $hP_{107-115}$. They inhibited the growth of all bacterial strains investigated and showed antifungal activity against *C. albicans*. Outer and inner membrane permeabilization studies, as well as measurements of transmembrane electrochemical potential provided evidence that HLH peptides and their C-terminal helix domains, $cP_{107-114}$ and $hP_{107-115}$ respectively, kill Gram-negative bacteria by crossing the outer membrane and subsequently causing damage to the inner membrane through pore formation.

Conclusion and Future Prospects

Ovotransferrin together with lysozyme are known as the major antimicrobial components in the defense system of avian egg albumin. Despite their structural properties are well characterized, the physiological roles as well as the antimicrobial mechanism of their actions remained mystery for decades. The antimicrobial activity of ovotransferrin has been believed to be due to its ability to bind and sequester iron from the egg-white thus rendering it an iron-deficient environment for microbial growth. Recent work from our laboratory and in the literatures provided a strong evidence that ovotransferrin can kill bacteria and that its bactericidal activity is completely independent of its iron-sequestration properties (Ibrahim, 1997b; Ibrahim et al., 1998; Ibrahim et al., 2000; Tranter and Board, 1982; Tranter and Board, 1984). In parallel, concrete genetic and biochemical evidence that antimicrobial action of lysozyme is independent of its catalytic function has also been presented (Ibrahim, 1998; Ibrahim et al., 1996a; Ibrahim et al., 1996b; Ibrahim et al., 2001a). This functional mystery of the two antimicrobial proteins, ovotransferrin and lysozyme, in the avian egg albumin tend to reflect complex molecular interconversion defence strategy. Most likely the avian egg defence system operates in a similar fashion like innate immunity systems generally distributed in nature by employing small antimicrobial peptides.

Antibacterial peptides are widespread in nature and have been found to play crucial role in natural defense systems including vertebrates, invertebrates, plants and bacteria (Boman, 1995). Expression of their genes is induced by bacterial infection.

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Antimicrobial peptides of mammalian and insect innate defence systems, such as defensin-like peptides, are basically synthesized as a large preprotein of ~95 Da. This precursor is processed by specific proteases to produce the mature bactericidal peptide (Boman, 1995; Lamberty et al., 1999; Lehrer et al., 1991). Our study demonstrates the presence of cationic antimicrobial peptides/domains within the sequences of both ovotransferrin and lysozyme molecules. Of special interest is the structural motif of these antibacterial peptides, though different from each other, shows remarkable structure similarities with known bactericidal defensins. Numerous defensins have been characterized from various forms of life, but for the first time defensin-like peptides have been found in avian egg albumin. The helix-loop-helix (HLH) antimicrobial peptide found in lysozyme (Ibrahim et al., 2002; Ibrahim et al., 2001b) resembles known motifs commonly found in membrane-active peptides including cecropins (Boman et al., 1991). On the other hand, the helix-sheet antimicrobial peptide found in ovotransferrin exhibits structure motif similarity with insect defensins (Fig. 3) (Lamberty et al., 1999). Identifying common Asp-X sequence at loop regions of both lysozyme (Fig. 4) and N-lobe of ovotransferrin (Fig. 1), surrounding their newly discovered defensin-like peptides, paves the way to future studies on clarifying a possible common defense mechanism of avian egg albumin which is a proteinase-deficient environment. Furthermore, the results herald a fascinating opportunity to the potential use of these peptides in therapy and infant formulas. Eventually, our results demonstrated the unique location of these surface exposed bactericidal domains on either the exterior lip of the N-lobe of OTf or at the upper lip of the active site of Lz, thus raised the tantalizing question of whether these domains have other biological function(s) in the egg albumin.

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CONTENTS

SIMULTANEOUS PILOT-SCALE EXTRACTION AND PRECIPITATION OF EGG YOLK PHOSPHOLIPIDS USING SUPERCRITICAL TECHNIQUES.

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Keywords: yolk, supercritical fluid, phospholipids

Abstract

Egg yolk is an excellent natural emulsifier, widely used in food industry. The emulsifying properties of egg yolk are based on the large proportion of phospholipids in egg yolk lipids. Egg yolk powder contains about 60 % of lipids, constituted of neutral lipids (65 %), phospholipids (31 %) and cholesterol (4 %).
Utilization of supercritical fluid techniques (SF-techniques) is a modern way to selectively extract and fractionate non-polar and slightly polar components from foods and food products.
In this study we created a method to precipitate the egg yolk phospholipids by using both supercritical extraction and supercritical antisolvent techniques.

Résumé

Le jaune d'œuf est un excellent émulsifiant naturel, largement utilisé dans l'industrie agroalimentaire. Les propriétés émulsifiantes du jaune sont basées sur sa forte proportion de phospholipides. La poudre de jaune contient environ 60 % de lipides constitués de lipides neutres (65 %), de phospholipides (31 %) et de cholestérol (4 %).
L'utilisation de techniques par fluide supercritique (FS) est une façon moderne d'extraire sélectivement et de fractionner les composés non polaires ou peu polaires contenus dans les aliments. Dans cette étude, nous avons créé une méthode pour précipiter les phospholipides du jaune d'œuf en utilisant des techniques d'extraction par fluide supercritique.

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Introduction

In laboratory conditions, lipid fractions of natural materials are usually extracted by using a different kind of combinations of organic solvents. However, if the lipids are intended to human purposes, the possible residues of the organic solvents may limit the use of the isolated lipid fractions. So, alternative techniques for the separation of lipids are mostly recommended.

When a gas is compressed and reaches a certain pressure, it starts to turn to liquid. If the gas is simultaneously heated above a certain temperature, no amount of compression of the hot gas will cause it become to liquid. This temperature is named as critical temperature and the corresponding pressure as critical pressure. These values are dependent on the properties of the substance used and the state of the substance is called supercritical fluid. The use of supercritical fluid in different kind of applications is a constantly increasing area on the field of separation technologies. Probably, the most common and most well-known application is the supercritical fluid extraction (SFE). The industrial applications of SFE-techniques are concentrated on tobacco extraction, making of spice extracts and extraction of fats and oils (Sihvonen et al. 1999).

Recently, supercritical fluids have been started to utilise also as an antisolvent. Especially, the micronization of pharmaceutical compounds is a continuously developing area of supercritical antisolvent processes. A large group of acronyms for these techniques has been launched. Some examples are shown in Figure 1.

Egg yolk powder contains about 60 % of lipids, constituted of neutral lipids (65 %), phospholipids (31 %) and cholesterol (4 %). Egg yolk is an excellent natural emulsifier. The emulsifying properties of egg yolk are based on the large proportion of phospholipids, which are an important component of egg yolk lipoproteins. They contain both hydrophilic head groups and lipophilic fatty acid groups. The major component of egg yolk phospholipid is phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Egg yolk phospholipids are widely used in food industry, cosmetics and pharmaceutical applications. (Sugino et al 1997).

Neutral lipid and cholesterol fractions of egg yolk are soluble in supercritical carbon dioxide (SC-CO_2), whereas the phospholipids are soluble at very high pressures, thus usually considered insoluble. Some studies have presented the separation of different lipid fractions from the egg yolk. Bringe (1997) removed 74 % of the fat and 90 % of the cholesterol by using SC-CO_2. Boselli and Caboni (2000) extracted phospholipids from dried egg yolk using microscale neat supercritical carbon dioxide system.

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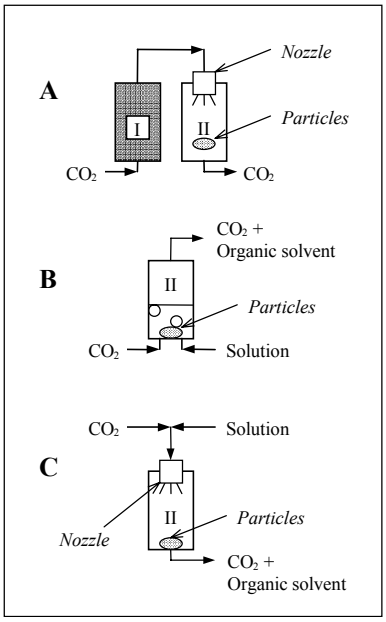

Figure 1: Typical examples of SF micronization processes (Sihvonen et al., 1999).
A=RESS - Rapid Expansion of Supercritical Solution
B=PGSS - Particles from Gas-Saturated Solution)
C=SAS - Supercritical Anti-Solvent

In this study we have developed a pilot scale method to separate the egg yolk phospholipids from egg yolk powder by using both supercritical extraction and supercritical antisolvent micronization techniques.

Material and Methods

Egg yolk powder was purchased from Scanegg Suomi Ltd. The powder was stored according to the recommendations of the manufacturer.

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The basic compositions of the different fractions were determined according to the following methods:
The nitrogen content of the samples was determined by using Kjeltec Auto 1030 Analyzer. The protein content was calculated by multiplying Kjeldahl nitrogen by factor 6,25.
The content of total carbohydrates was calculated with formula:
Total carbohydrates (% / FW) = 100 – moisture (%) – protein content (% / FW) – crude fat (% / FW) – ash (% / FW)) = total carbohydrates (g / 100 g FW)
(FW = fresh weight of the sample)
Fat content of the samples was determined with the Twisselman method (AOAC 1980).
Ash content was analysed by weighing before and after burning the samples at 600 °C.
The energy content was calculated with following factors: protein 17 kJ/g; fat 37 kJ/g; carbohydrates 17 kJ.
The phospholipid classes of PLS-product were analysed by HPLC according to the method of Kivini et al. (2003).
The method consisted of three separated steps: SF-extraction (isolation of neutral lipids), SF-ethanol -extraction (isolation of slightly polar lipids) and precipitation with antisolvent technique. The pilot scale apparatus used in this study consists of an extraction chamber, precipitation (crystallization) chamber and a separator. The detailed description of the apparatus used is given in Adami et al. (2003).
SF - extraction
The extraction chamber was filled with egg yolk powder (2000 g). Before the extraction the egg yolk powder was mechanically granulated as described in Aro et al. (2002).
The extraction chamber is pressurized with carbon dioxide. When the operating pressure is reached, the carbon dioxide is allowed to flow through the dry egg yolk in the extractor. The pressure, flow rate and the temperature of the CO_2 are monitored continuously.
Extraction was carried out using pure CO_2 in 70°C and 450 bar. The total extraction time was 3 hours. The quantity of the extract (NLs; eg. neutral lipids, cholesterol and moisture) was measured.
SF – ethanol - extraction
In the second step, the SC-CO_2 was modified with 96 % ethanol. The tested amounts of ethanol varied: 5-15 %, and the corresponding extraction times were 3-8 hours. SF – ethanol extraction was carried out in 70°C and 400 bar. The ethanol solution of slightly polar lipids was collected and concentrated.
Precipitation with antisolvent technique
In the final step the pressure of the extractor was set to be 350 bar, the pressure of the precipitation unit 270 bar, and the temperature in the both chambers was 70 °C. When these operative conditions were reached, the ethanol solution of the lipid fraction to be

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precipitated is pumped with a high pressure liquid pump via the extraction chamber to the precipitation chamber. The lipid fraction is precipitated on the walls of the chamber, and the ethanol is collected to the separator.
When the ethanol solution has been delivered, the liquid pump is stopped, and only CO_2 continues to flow through the chamber. Finally, CO_2 flow is stopped, the system is depressurized down to the atmospheric pressure and the chamber is opened. The precipitated phospholipid fraction (PLS-product) is collected from the walls of the chamber.

Results and discussion

The basic composition of the egg yolk powder is presented in table 1.

Table 1: The composition of the egg yolk powder

Substance	**Powder before extraction g/100 g fw**
Protein	35,9
Carbohydrates	0,04
Fat	52,9
Moisture	4,46
Ash	6,7
Energy (kJ)	2568

The SC-CO_2 -extraction from granulated egg yolk powder (2000 g) produced about 800 g of NLs in 3 hours. After the $SFCO_2$ –EtOH –extraction, concentration and precipitation, about 80 g of polar lipids (mostly phospholipids, PLS) was recovered from the walls of the precipitation chamber.
In the phospholipid analysis the results showed that the PLS-product contained 720-780 mg/g phosphatidylcholine and 120 –160 mg/g phosphatidylethanolamine.
A non-toxic pilot scale method for the preparation of egg yolk phospholipids by using supercritical techniques was developed in this study. The final phospholipid product shows high purity and good suitability to industrial processes. The possibilities to utilize this kind technique in industrial scale are under continous screening.

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CONTENTS

MODIFICATION OF LYSOZYME BY THE MEMBRANE TECHNIQUES

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Keywords: lysozyme monomer, lysozyme dimer, membrane techniques, ultrafiltration, hydrolytic activity

Abstract

The aim of the present work was to obtain lysozyme preparation with increased occurrence of enzyme polymeric forms by using membrane techniques (ultrafiltration -UF and reverse osmosis – RO). The influence of selected parameters of the membrane process on the extent of enzyme modification was examined. The study showed, that membrane technique developed for the production of lysozyme monomer, could also be used to a direct production of preparation containing enzyme polymers. In the optimal conditions of the modification procedure the obtained preparation contained 53,3% of lysozyme polymeric forms.

Résumé

L'objectif de cette recherche était d'obtenir du lysozyme avec un enrichissement en formes polymériques en utilisant des techniques à membranes (ultrafiltration et osmose reverse). L'influence des paramètres sélectionnés sur l'étendue de la modification de l'enzyme a été examinée. La technique que nous avons développée pour la production du monomère peut aussi être utilisée pour une production directe de formes polymériques du lysozyme. Dans les conditions optimales, les préparations contiennent 53,3% de formes polymériques du lysozyme.

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Introduction

Lysozyme (E.C.3.2.1.17) is the enzyme which catalyses the hydrolysis of specific polysaccharides being components of bacteria cell wall. Its characteristic feature is the ability to form complexes with other proteins and formation of biopolymers (Jolles and Jolles, 1984; Proctor and Cunningham, 1988). Another important trait of that enzyme is its association capacity (Sophianopoulos and Holde, 1964). Lysozyme monomer demonstrates strong antibacterial potential against Gram-positive bacteria. That phenomenon has found practical application in the food and pharmaceutical industries as well as in medicine (Akashi, 1972, Proctor and Cunningham, 1988, Cąkała 1994, Garbuliński, 1994, Kijowski and Lesnierowski, 1995). The spectrum of lysozyme practical use can be substantially broadened by its modification. Numerous authors have reported that by dimerisation a novel, specific enzyme activity can be reached against the Gram-negative bacteria without any loss in the bacteriacidal properties against the Gram-positive bacteria (Nakamura *et al.*, 1991; Ibrahim *et al.*, 1991; Nakamura *et al.,* 1992, Kiczka, 1994; Kijowski *et al.*, 2000). At the present moment the basic research topic is the development of effective modification techniques of lysozyme aimed at the extension of its activity spectrum. Such studies have been carried out in certain research units throughout the world (Nakamura *et al.*, 1991; Ibrahim *et al.*, 1991; Ibrahim *et al.,* 1994, Ibrahim *et al.*, 1996; Kijowski, *et al.,* 1998, Kijowski, *et al.,* 1999; Lesnierowski, *et al.,* 2001). During our investigations concerning lysozyme isolation from chicken egg white by the membrane technique we found small quantities of polymerised lysozyme in the ultrafiltration module (Kijowski *et al.*, 2000). The authors suggested that observation could also be used in the effective modification of lysozyme. The purpose of the present study was to investigate that phenomenon and to use the membrane technique in the production of polymerised enzyme

Material and Methods

Raw material. Lysozyme obtained from albumin of fresh laid chicken eggs of ASTRA S layers kept in the breeding farm from the Institute of Zootechnic Broiler Breeding Farm at Zakrzewo near Poznan was taken for experiments. The ion exchange technique was used to isolation of lysozyme from the egg white (Lesnierowski, 1997).
Lysozyme modification by the membrane technique. The enzyme was modified by the membrane technique in the DDS 20-0.36 LAB ultrafiltration plate module under defined conditions of pressure (15, 20 and 30 bar), temperature (20^0, 35^0 and 50^0C) and time (1.0; 2.0 and 5.0 h). Membranes type HR95PP of Uniq Filtration Co

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(Denmark) was used. The obtained lysozyme solution was dried thereafter in the B-191 Bűchi Spray Drier.

Analysis of the basic composition of enzyme preparations. Crude protein content was determined by Kjeldahl method, sodium chloride by Mohr method, moisture by drying to constant weight.

Hydrolytic activity. The lysozyme activity was determined by means of the spectrophotometic method based on the lysis of the cell wall of *Micrococcus lysodeikticus* by the enzyme (Lesnierowski and Kijowski, 1995).

Electrophoresis. The analysis was conducted using the SDS-PAGE method (Laemmli, 1970; Lesnierowski, 1997). The percentages of individual forms of the protein were assessed densimetrically using the QuantiScan 2.0 software by Biosoft.

Statistical analysis. The results were subjected to statistical analysis using the STATISTICA PL version 6.0 software. The average value, minimum, maximum, standard deviation, standard error and confidence interval were calculated for each variable. The significance of results was verified with the use of the Schaffe test.The one-factor analysis of variance (ANOVA) was conducted. Curves of regression and the Pearson coefficient of correlation were determined.

Results and discussion

The study described here comprised the determination of the effects of time, pressure and temperature on the degree of lysozyme polymerisation during modification by the membrane technique in the ultrafiltration module. The solution of lysozyme was subjected to diafiltration in the DDS 20-036 LAB plate ultrafiltration module under controlled parameters of the examined factors Lysozyme of our own production obtained by the ion exchange method was used as experimental material. The hydrolytic activity of enzyme prior to modification amounted to around 16.000 U/mg, the protein content was at the level 78 to 75%, sodium chloride 10 to 15% and sample moisture content 8 to 9%. The obtained preparations demonstrated various amounts of lysozyme polymeric forms, however, their basic composition (crude protein, sodium chloride, H_2O) was found stable and similar to the composition of monomer.

Time and pressure. In the first stage of study the effect of pressure and time on lysozyme modification by the membrane technique was examined. The experiment was conducted in a ultrafiltration module at 15, 10 and 39 Ba, during 1.0, 2.0 and 5.0 hours under constant pH 7 and constant temperature of 20^0C. The experimental results are shown in the Table 1.

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The obtained enzyme preparations contained various forms of polymerised lysozyme. The present results confirmed the earlier findings of coupling the enzyme molecules into larger agglomerates, when using the membrane technique

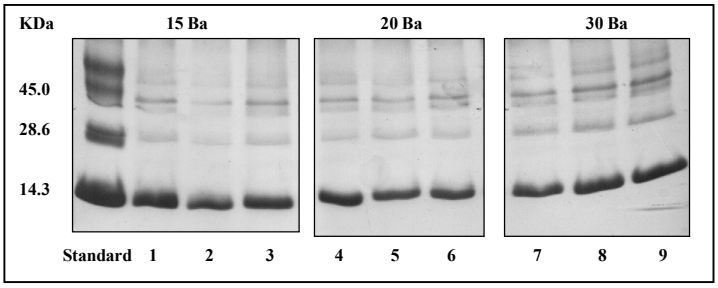
Table 1. The quantities of polymeric forms and hydrolytic activity of lysozyme preparations obtained during modification by the membrane technique for 1.0; 2.5 and 5.0 h at 15, 20 and 30 Ba

Sample	*Time h*	*Temp. °C*	*Pres. Ba*	*pH*	*Monomer %*	*Dimer %*	*Trimer %*	*Activity U/mg*
1	1.0	20	15	7.0	74.1[a]	16.5[a]	9.5[a]	11444[f]
2	2.0	20	15	7.0	73.0[a]	16.6[a]	10.6[a]	10049[e]
3	5.0	20	15	7.0	72.1[a]	17.6[b]	10.3[a]	6842[b]
4	1.0	20	20	7.0	69.2[b]	17.5[b]	13.4[b]	7924[d]
5	2.0	20	20	7.0	67.9[b]	17.8[b]	14.3[b]	7086[c]
6	5.0	20	20	7.0	67.5[b]	18.1[b]	14.5[b]	6119[a]
7	1.0	20	30	7.0	69.0[b]	17.0[b]	14.1[b]	6949[bc]
8	2.0	20	30	7.0	68.2[b]	17.5[b]	14.2[b]	6055[a]
9	5.0	20	30	7.0	67.7[b]	17.7[b]	14.6[b]	5913[a]

a-f: different superscripts in the columns denote significant difference at $p < 0.05$

for isolation of enzyme from chicken egg white (Lesnierowski and Kijowski, 2000). Lysozyme polymeric forms and their quantities in the preparations were examined by the electrophoretic method. The electrophoregrams on Figure 1 present protein bands, which correspond to monomer, dimer and trimer of the lysozyme. In the applied modification conditions monomer was the dominant fraction (around 70%), while the content of dimer amounted to 17 – 18% and of trimer to 10 – 14%.

Figure 1. Electrophoretic analysis of lysozyme modified by the membrane technique for 1.0; 2.5 and 5.0 h at 15, 20 and 30 Ba



1,4,7 – samples modified 1.0 h
2,5,6- samples modified 2.0 h
3,6,9 - samples modified 5.0 h

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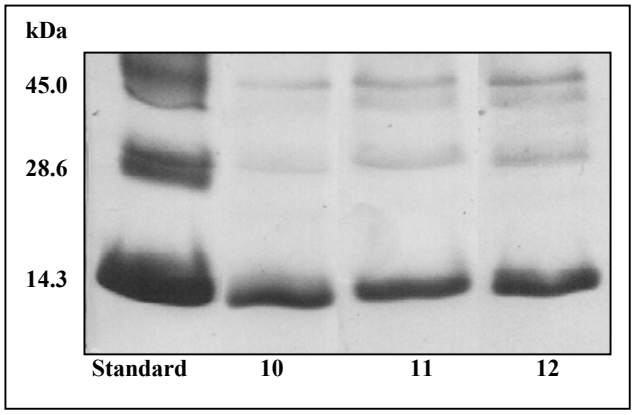
The statistical analysis of the effect of modification time on the degree of enzyme polymerisation demonstrated no significant differences in the quantity of dimer and trimer fractions. Only in the sample obtained under 15 Ba pressure and modified for 5.0 h the amount of dimer was significantly different from the two other samples modified for 1.0 and 2.0 h. In the case of the other samples it can only be said about a tendency to the increase of both dimer and trimer quantities resulting from the extended modification time.The statistical analysis revealed that to obtain the highest quantity of dimer the modification time has to amount to at least 2.0 h under the pressure of 20. The further study was carried out under such parameters of pressure and time.

Temperature. The experiments on the effect of temperature on the effectiveness of enzyme polymerisation were conducted in the temperature range from 20 to 50^0C. Heating of samples during the membrane process increased the effectiveness of lysozyme polymerisation as indicated by the results of the electrophoretic analysis (Figure 2).

Figure 2. Electrophoretic analysis of lysozyme preparation modified at 20, 35 and 50^0C



10 – sample modified at 20°C
11 - sample modified at 35°C
12 - sample modified at 50°C

The obtained electrophoregrams demonstrated an increase of protein bands intensity corresponding to the dimer and trimer of lysozyme in the samples modified at higher temperature (pathways 10,11,12).

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The computerised densitometric analysis of the electrophoregrams contributed to a precise determination of the polymeric forms of lysozyme in the examined preparations (Table2).

Table 2. The quantities of the polymeric forms and hydrolytic activity of enzyme modified by the membrane technique at 20, 35 and 50^0C

Sample	*Temp. °C*	*Pres.. Ba*	*pH*	*Monomer %*	*Dimer %*	*Trimer %*	*Activity U/mg*
10	20	20	7.0	68.2^a	17.8^a	14.1^a	7043^a
11	35	20	7.0	58.1^b	23.0^b	18.7^b	6961^a
12	50	20	7.0	46.7^c	33.2^c	20.1^c	5940^b

a-b: different superscripts in the columns denote significant difference at $p < 0.05$

The experimental results demonstrated that the elevation of modification temperature from 20^0C to 50^0C was followed by an increase of the dimeric form of enzyme by 80% (from 18 to 33%) and of the trimeric by 43% (from 14 to 20%) in the preparation. The statistical analysis of results confirmed the significant effect of modification temperature of lysozyme on the degree of polymerisation.

The analysis of the experimental findings has revealed that temperature is a very significant factor influencing the effectiveness of lysozyme modification with the membrane technique. Heating of enzyme samples during modification was followed by substantial increase of dimer and trimer quantities in the preparations. Similar phenomenon was observed in the studies on thermal modification of enzyme (Ibrahim *et.al...* 1996; Kijowski *et al.*. 2000). It could be expected that elevation of temperature to the level of 70 – 80^0C would more favourably influence modification effectiveness. However the used apparatus and membranes limited the temperature level. In the available ultrafiltration module the maximum permitted temperature attained 50^0C.

Conclusion

The modification of lysozyme by the membrane technique presented in this study is an effective tool that can be used in the direct production of lysozyme preparation of an increased quantity of the polymeric forms. Temperature pressure and time of modification as well as the acidity of medium significantly affected the quantity of polymers in the obtained preparations. At the optimal modification conditions *i.e.* 20 Ba pressure, 50^0C temperature and 2.0 h modification time the produced lysozyme

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preparation contained 53.3% polymeric forms in total. *i.e.* 33.2% of dimer and 20.1% of trimer.
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ANTIBACTERIAL ACTION OF LYSOZYME MODIFIED BY THE ULTRAFILTRATION TECHNIQUE

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Keywords: lysozyme monomer, lysozyme dimer, ultrafiltration, antibacterial activity

Abstract

The effectiveness of antibacterial action of lysozyme modified by the membrane technique (ultrafiltration and reverse osmosis) against selected strains of bacteria was determined. Its bacteriostatic activity was dependent on modification conditions. Among lysozyme preparations modified by ultrafiltration the highest bacteriostatic activity against selected strains of *Proteus mirabilis, Pseudomonas fluorescens* and *Staphylococcus epidermidis* bacteria was noted in the preparation containing 53,3% polymeric forms. The modification procedure facilitates the extension of antibacterial spectrum of lysozyme, particularly against *Pseudomonas fluorescens* and *Proteus mirabilis* Gram (-) bacteria.

Résumé

L'efficacité de l'action antibactérienne du lysozyme modifié par des techniques membranaires (ultrafiltration et osmose reverse) contre des souches de bactéries sélectionnées a été déterminée. L'activité bactériostatique dépend des conditions de procédé. Parmi les préparations de lysozyme modifiées par ultrafiltration, l'activité bactériostatique la plus importante contre des souches de *Proteus mirabilis, Pseudomonas fluorescens* et *staphylococcus epidermis* a été obtenue avec la préparation contenant 53,3% de formes polymériques. La procédure de modification facilité l'extension du spectre antibactérien du lysozyme, particulièrement contre *Pseudomonas fluorescens* et *Proteus mirabilis*, bactéries Gram (-).

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Introduction

Lysozyme as the enzyme of antibacterial properties has been used both in the pharmaceutical and food industries. Lysozyme addition has been found to extend the storage life of meat and meat products. Lysozyme plays a particular role in cheese ageing by the reduction of butyric fermentation bacteria (*Clostridium tyrobutyricum, Clostridium butyricum*), which adversely affect cheese quality. It has been found that the spectrum of lysozyme antibacterial action can be broadened by its modification and formation of polymeric forms (Hughey and Johnson, 1987; Proctor and Cunningham, 1988; Johnson, 1994; Kiczka, 1994; Danyluk and Kijowski, 2001; Kijowski et al., 2002).

The range of lysozyme activity can be extended by modifications leading to changes in the conformation of enzyme molecules and resulting in the production of its polymeric forms. It was found that a modified form of lysozyme shows a significantly wider bacteriostatic activity, including Gram-negative bacteria, than native lysozyme. It has to be emphasized that the modified enzyme retains antibacterial activity against Gram-positive bacteria, characteristic for the monomer (Ibrahim et al., 1991; Ibrahim et al.,1994; Ibrahim et al., 1996; Kijowski et al., 2000; Pellegrini et al., 1992; Proctor and Cunnigham, 1998). Any modification of lysozyme properties that could render useful in the case of Gram-negative and Gram-positive bacteria would be an important feature.

Lysozyme can be lethal to Gram-negative bacteria if the interaction with the bacterial membrane is strengthened by modifying the enzyme surface hydrophobicity, e.g. chemically modified with palmitate or stearate residues (Ibrahim et al., 1991, Ibrahim et al., 1993) or genetically used with a hydrophobic pentapeptide (Ibrahim et al.,1994; Kato et al., 1998). All these derivatives exhibit strong bactericidal action against *E.coli* K12. They are capable to insert into lipid layer and subsequently disrupt the electrochemical potential by forming ion pores in the cell membrane. The data suggest that lysozyme molecule can be lethal to bacteria if its structure supplies a hydrophobic domain on the molecule surface.

Material and Methods

Lysozyme obtained from chicken egg white by the ion exchange method developed by Leśnierowski (1997) in the Poultry Products Technology of the Agricultural University in Poznań was used in experiments. The modification of lysozyme solution by the membrane technique was carried out in the ultrafiltration plate module DDS 20-0.36 LAB at 20Ba, pressure of 50°C temperature and pH 7.0, 8.0 and 9.0. Uniq

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Filtration membranes HR95PP were used. During the modification procedure the thermostat LW102 AURITRONIC kept the temperature at the required level. The obtained lysozyme solutions were spray-dried in Bűchi B-191 Spray Drier (Lesnierowski and Kijowski, 2000; Lesnierowski and Kijowski, 2001).
Both the lysozyme monomer and lysozyme preparations modified by ultrafiltration were used in microbiological tests to compare their effectiveness against selected bacteria strains. The details on modified lysozyme preparations used in the microbiological tests are presented in Table 1 where the enzyme activity, the quantities of monomer and polymer forms in the preparations and the modification parameters are specified. The hydrolytic activity was measured using the spectrophotometric method based on the application of the phenomenon of *Micrococcus lysodeickticus* cell wall decomposition by lysozyme (Leśnierowski and Kijowski, 1995). The presence and quantities of the polymeric forms in the enzyme preparations were examined by the electrophoretic technique using SE-600 apparatus of Hoefer Scientific Instruments and the SDS-PAGE method (Laemmli, 1970; Lesnierowski, 1997).

Table 1 Parameters of lysozyme preparations modification by membrane technique

Lysozyme symbol	***Enzyme activity [U/mg białka]***	***Content of monomer [%]***	***Content of dimer [%]***	***Content of trimer [%]***	***Modification parameters***		
					Temperature [^{0}C]	pH	Pressure [Ba]
M1	6443	46,7	32,7	20,6	50	7	20
M2	4530	65,5	30,5	6,0	50	8	20
M3	5000	54,9	29,0	16,1	50	9	20

Gram (+) and Gram (-) bacteria strains: (*Staphylococcus epidermidis ATCC 12228 nr IW 1514, Proteus mirabilis nr IW 513, Pseudomonas fluorescens NCTC 3756 nr IW 1095)* characteristic for poultry meat were used in the experiments. They were delivered by the National Veterinary Research Institute in Puławy (National Collection of Bacterial Strains) and by the Institute of Immunology and Experimental Therapy in Wrocław.
Lysozyme concentrations of 2.5 mg/ml and 5 mg/ml were used. The solution of the required lysozyme concentration was prepared by dissolving the dry lysozyme in sterile distilled water. From 24 h old bacteria culture a series of dilutions was prepared and 1 ml of bacteria suspension was transferred to test tubes with 9 ml of NaCl physiological solution. One ml of bacteria suspension from the respective dilution was

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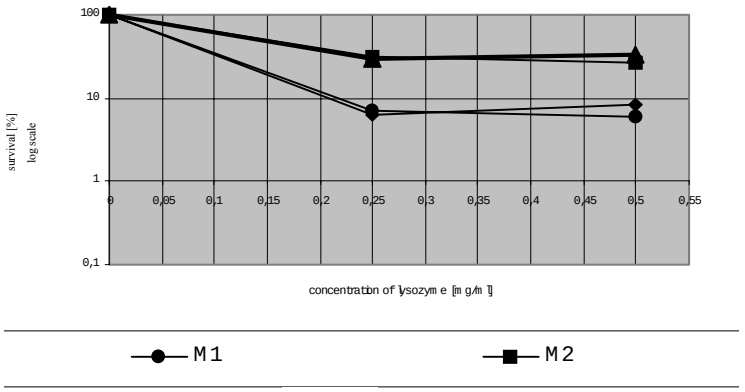
added to the test tubes each containing 4 ml of NaCl physiological solution and 5 ml of lysozyme solution. Inoculation of bacteria was carried out onto OXOID culture media: Agar, Mannitol Salt Agar, Pseudomonas Agar Base and MacConkey. Each time lysozyme sterility was examined.

Results and discussion

Experiments were conducted with the use of modified membrane technique to obtain lysozyme preparations under optimal modification parameters reported in the earlier studies. Among the examined parameters such as temperature, pH and pressure, the variable parameter was pH (Table 1).
The increase of pH value was found to influence negatively the effectiveness of enzyme polymerisation and at pH 9.0 the lowest quantity of lysozyme dimer was obtained. It was noted that all modified lysozyme preparations demonstrated antibacterial action dependent on kind of bacteria and the lysozyme preparation used.
The tests with *Proteus mirabilis* bacteria concerning the antibacterial action of lysozyme preparations modified by the ultrafiltration technique revealed that M1 lysozyme containing the highest quantity of dimer was most effective. (Figure 1). Similar effective action was demonstrated with M3 lysozyme preparation. On the other hand, the lowest antibacterial effectiveness in the group of lysozyme preparations modified by ultrafiltration was found in M2 preparation which contained the greatest quantity of monomer and showed the lowest hydrolytic activity. It was also observed that the use of higher concentration of lysozyme had no effect on reduction of *Proteus mirabilis* survival.

Figure 1 The effect of lysozyme preparations on survival of *Proteus mirabilis* bacteria



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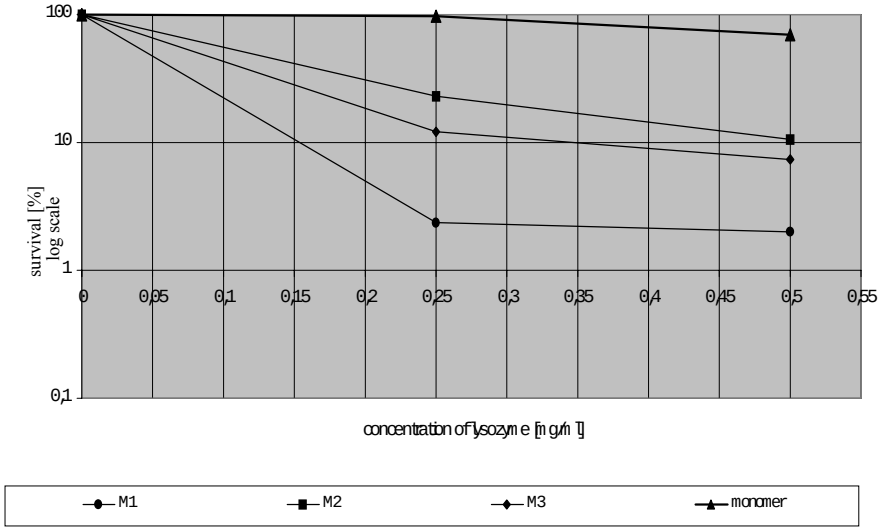
M1 lysozyme preparation also exhibited particularly effective antibacterial action against *Pseudomonas fluorescent* bacteria. The use of M2 and M3 lysozyme preparations in comparison with the lysozyme monomer also contributed to reduction of *Pseudomonas fluorescent* survival (Figure 2). The used monomer was found ineffective in the reduction of *Proteus mirabilis and Pseudomonas fluorescent* Gram (-) bacteria number.

Figure 2 The effect of lysozyme preparations on survival of *Pseudomonas fluorescens* bacteria



The influence of lysozyme preparations, modified by the membrane technique, on S*taphylococcus epidermdis* Gram (+) bacteria showed that M1 enzyme preparation was found most effective similarly as in the case of Gram (-) bacteria (Figure 3).
It was found , however, that lysozyme monomer exhibited substantially greater effectiveness against *Staphylococcus epidermidis* within Gram (+) bacteria in comparison with the other examined bacteria strains of Gram (-) group. On the other hand, a more effective action was observed with some modified lysozyme preparations, e.g. M1. Lysozyme preparations of higher content of M2 and M3 monomer showed antibacterial action similar to that of lysozyme monomer.
Among lysozyme preparations modified by the membrane technique the greatest reduction of the examined bacteria species was noted with M1 preparation which contained the highest quantity of dimer and demonstrated the highest hydrolytic activity (Table1).

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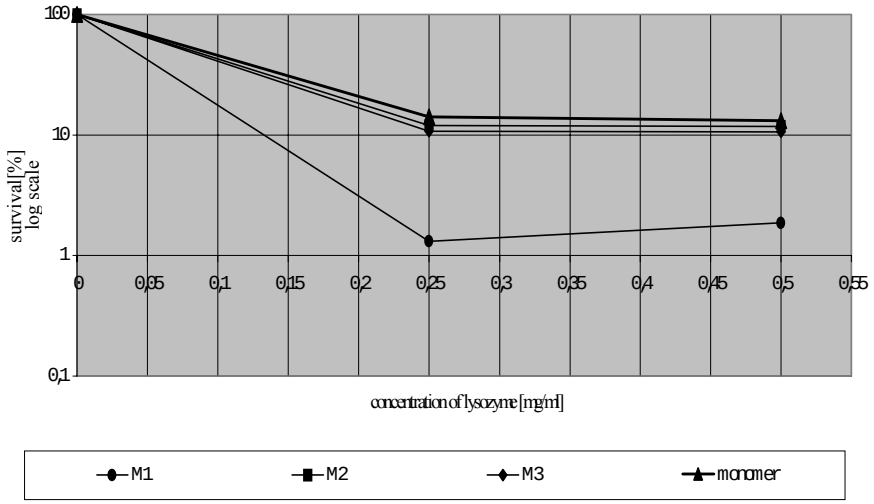
Figure 3 The effect of lysozyme preparations on survival of *Staphylococcus epidermidis* bacteria



The observed increase in antibacterial activity of modified lysozyme preparations against Gram (-) bacteria was not associated with a decrease of that activity against Gram (+) bacteria. The experiments have shown that the used preparations of lysozyme demonstrate different activity being dependent on the kind of bacteria. Modification of lysozyme by the membrane technique broadened the spectrum of enzyme antibacterial action especially against *Pseudomonas fluorescens* and *Proteus mirabilis* Gram (+) bacteria. In the case of Gram (-) bacteria the most effective antibacterial action was noted with lysozyme preparation containing the highest quantity of dimer and the lowest of monomer.
It seems justified to investigate the antibacterial activity of lysozyme preparation of low monomer content against a greater number of various bacteria strains.
This study was financially supported by the KBN grant no. PO6GO3818

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CONTENTS

OVOMUCIN PURIFICATION: SOME PRELIMINARY STUDIES TO MODIFY THE ISOELECTRIC PRECIPITATION METHOD

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Keywords: ovomucin, purification, isoelectric precipitation,
gel filtration chromatography

Abstract

In the first trial ovomucin was purified by the isoelectric precipitation method. We used a sieve with a very narrow square aperture instead of centrifugation to collect ovomucin. We found that by using a sieve the yield of ovomucin was about 90% of the amount obtained by centrifugation. When collected on a sieve, ovomucin is not so tightly packed as after centrifugation and, thus, can be solubilized more easily without any mechanical treatments. The sieve method is also very quick and easy to upscale without high costs.
In the second trial we further purified the ovomucin produced by the isoelectric precipitation method by using gel filtration chromatography. We obtained higher yields of the soluble ovomucin complex essentially free of other egg albumen proteins.

Résumé

Dans le premier essai, l'ovomucine était purifiée par précipitation isoélectrique. Un tamis dont les orifices sont étroits (140µm) plutôt que la centrifugation était utilisé pour la récupération de l'ovomucine. Le tamis permettait de récuperer 90% de la quantité d'ovomucine obtenue par centrifugation. Etant moins tassée par utilisation de tamis que par utilisation d'une séparation centrifuge, l'ovomucine était plus facilement solubilisée sans autre traitement mécanique. De plus, la séparation à l'aide d'un tamis est rapide, bon marché et facilement extrapolable à plus grande échelle.
Dans un second essai, l'ovomucine produite par précipitation isoélectrique était purifié par chromatographie en gel filtration. Les rendements d'extraction des complexes

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solubles d'ovomucine, essentiellement dépourvues des autres protéines du blanc d'œuf, étaient plus élevés.

Introduction

Ovomucin is an egg albumen protein representing 2-4% of the total egg albumen proteins. It is a potential ingredient for functional foods, as it has been reported to have antiviral, antitumour and hypocholesterolemic properties (Ohami et al., 1993; Tsuge et al., 1996, Nagaoka et al., 2002). Ovomucin is usually fractionated from egg albumen by using the isoelectric precipitation method (IEP) (Kato et al., 1970). The IEP method consists of a precipitation step (in which egg albumen is diluted with water, followed by acidification), a collection step (usually done by centrifugation) and a washing step(s) (ovomucin precipitate is repeatedly washed several times with water and 2% KCl). The gelatinous ovomucin precipitate thus obtained has very limited solubility in conventional buffer solutions and is also contaminated with other albumen proteins. Alternatively, ovomucin could be separated by using gel filtration chromatography (GFC) (Young and Gardner, 1970). An advantage of the GFC method compared to IEP is that the separated ovomucin is usually very pure (= free of other egg white proteins) and already in soluble form. The main disadvantage of GFC is a low yield of ovomucin due to its low content in egg white, and poor solubility of the ovomucin.
The aim of this preliminary study was to examine some alternative ways to improve the IEP method. To avoid ovomucin packing during the collection step, we tested the possibility of collecting ovomucin using a sieve. In the second part of trial we tested the idea of Hayakawa and Sato (1976) of removing the other egg white proteins from the ovomucin sample before gel filtration, with the aim of improving the ovomucin yield obtained by the GFC method.

Material and Methods

Sieve trial.
The eggs were obtained from the local henhouse (MTT Agrifood Research Finland, Animal feeding section). Albumen was separated from the yolk with a household yolk separator. Whole egg whites (chalazae cords removed) of 60 eggs were pooled and homogenized with a household mixer at low speed to avoid foaming. Ovomucin was isolated from the pooled egg white sample both by the IEP sieve (IEPS) and conventional IEP centrifugation (IEPC) methods. In both methods the precipitation step was same, i.e. homogenized albumen sample was diluted with three volumes of

de-ionized water, stirred 30 minutes and adjusted to pH 6 with 1 N HCl. Next, in the case of the IEPS method, ovomucin was collected by pouring the albumen mixture onto a sieve with a square aperture of very narrow (140 μm) width. The crude ovomucin precipitate remaining on the sieve was collected and lyophilized without any further washing steps. In the case of the IEPC method, ovomucin was collected by centrifugation (10 000 ×g, 10 min, RT). The crude ovomucin precipitate was then washed twice with water using centrifugation and freeze-dried. The crude ovomucin yields obtained by both IEP methods were estimated by weighing the lyophilized ovomucin precipitations.

IEP-GFC method.

The eggs were purchased from a local grocery store. The whole egg white sample of 15 eggs was prepared as described above. Ovomucin was isolated from 300 g of albumen by using the IEPC method as above. The ovomucin precipitate thus obtained was homogenized with a small amount of water with an Ultra Turrax. Homogenized ovomucin was dispersed into 1100 g of phosphate-buffered saline (PBS, pH 7.4) and stirred overnight. Undissolved ovomucin was separated by centrifugation (10 000 ×g, 20 min, RT), and the supernatant was further concentrated using the Minitan membrane filtration system (Millipore, Bedford, MA, USA) equipped with a 100 000 MW cut off Mini-Ultrasette tangential flow device (Pall Gelman Laboratory, Ann Arbor, MI, USA). An aliquot (150 ml) of the concentrated ovomucin sample was introduced to a Sephacryl S-500 HR 50/90 XK column adapted on the ÄKTAexplorer system (Amersham Biosciences, Uppsala, Sweden), and eluated with a PBS buffer (pH 7.4) at a flow rate of 8 ml/min. The eluate was monitored by a UV detector at 280 nm. Fractions of 10 ml were collected.

Subunit profiles.

The ovomucin samples were analyzed by using the Superose 6 HR 10/30 dual column GFC method described by Hiidenhovi et al. (2002).

Results and Discussion

Sieve trial.

The crude ovomucin precipitations obtained by the IEPC and IEPS methods also contained various amounts of other coprecipitated egg white proteins. The purity of the crude ovomucin samples was estimated by using Superose 6 HR GFC. Figure 1 shows a typical GFC chromatogram. GFC separated the crude ovomucin into eight peaks. According to gel filtration performed with egg white protein standards, peak 4 was identified as ovotransferrin, peak 6 as ovalbumin, peak 7 as ovomucoid, and peak 8 as lysozyme. Peak 5 contained unidentified protein(s), probably globulins. Peaks numbered 1-3 contained ovomucin subunits: peak 1 was named as β-ovomucin, peak 2

as α2-ovomucin, and peak 3 as α1-ovomucin, in accordance with the nomenclature of Itoh et al. (1987). The amounts of coprecipitated egg white proteins in the crude ovomucin samples were measured by using standard curves created for each protein (area of peak 5 was included into area of peak 6 [ovalbumin]). By subtracting the amounts of coprecipitated proteins in the crude ovomucin samples, we found that the purity of crude ovomucin produced by the IEPC method was 63%, whereas by the IEPS method its purity was 43%. The main reason for this difference was presumably that the crude ovomucin precipitate collected with the sieve was not washed any further, whereas after centrifugation there were two additional washing steps with water.

These purity factors were used to estimate the actual ovomucin contents in the crude ovomucin precipitates obtained by both IEP methods used. Thus, by the conventional IEPC method we obtained 440 mg of crude ovomucin from 100 g of whole egg white, whereas after correction, egg white was found to contain 280 mg of ovomucin. In the case of the IEPS method, 100 g of whole egg white was found to contain 580 mg of crude ovomucin, whereas after correction the amount was 250 mg of ovomucin. So, by using a sieve we obtained about 90% of ovomucin compared to the yield achieved by centrifugation. The IEPS ovomucin had a gel-like appearance and was readily soluble without any mechanical treatments. The IEPS method was also very simple to perform and could be easily upscaled without high costs. It could, thus, be used as an alternative for IEPC, but washing steps should also be added to decrease the amount of coprecipitated protein in IEPS crude ovomucin.

IEP-GFC method.

In previous studies in which ovomucin has been purified by GFC, the reported ovomucin yields have been quite low (5-10 mg) per GFC run (Adachi et al., 1973; Awadé et al., 1994). Of course, this is also an upscaling issue, but the main reason for the low yield is that samples for GFC are prepared by diluting egg white with buffer, thus leading to samples containing low amounts of ovomucin. Therefore, in this study we tested the idea used earlier by Hayakawa and Sato (1976) to increase the amount of ovomucin compared to other egg albumen proteins before the GFC purification step.

In this study we used the IEPC method as a first pre-purification step. From 300 g of whole egg white sample we obtained 1490 mg of crude ovomucin with 50% purity (measured by the Superose 6 HR GFC method). Thus, our crude ovomucin precipitate contained 745 mg of ovomucin (about 250 mg of ovomucin/100 g of egg white). This crude ovomucin obtained by the IEPC method was then dissolved in a PBS buffer (PBS was chosen because this buffer can be used in many bioactive measurements). After stirring overnight, the insoluble materials were collected by centrifugation, washed twice with water by centrifugation, and lyophilized. The Superose 6 HR GFC analysis showed that this precipitate contained (insoluble) ovomucin with a purity of 99%. The supernatant (1120 ml) containing dissolved ovomucin was further concentrated to 340 ml by using 100 000 MW cut off membrane filtration. Besides

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increasing the ovomucin concentration in the sample, this step also decreased the amounts of coprecipitated egg white proteins with MW's under 100 000 kDa. A volume of 150 ml of concentrated ovomucin sample was loaded onto a Sephacryl S-500 HR column. The proteins in the ovomucin sample were eluated in two peaks, A and B (Figure 2). Peak A contained ovomucin, and peak B other egg white proteins. The fractions corresponding to peak A were pooled (220 ml), and a volume of 100 ml of the pooled fractions was dialyzed against water, lyophilized, and weighed (50 mg). About 110 mg of ovomucin was purified in one GFC run. The Superose 6 HR chromatogram revealed that the soluble ovomucin fraction appeared to be almost totally free of other egg white proteins (its purity was calculated to be 97%). Thus, by using this IEF-GFC method it was possible to obtain a soluble ovomucin sample (about 80 mg /100 g of egg white) ready for bioactivity studies.

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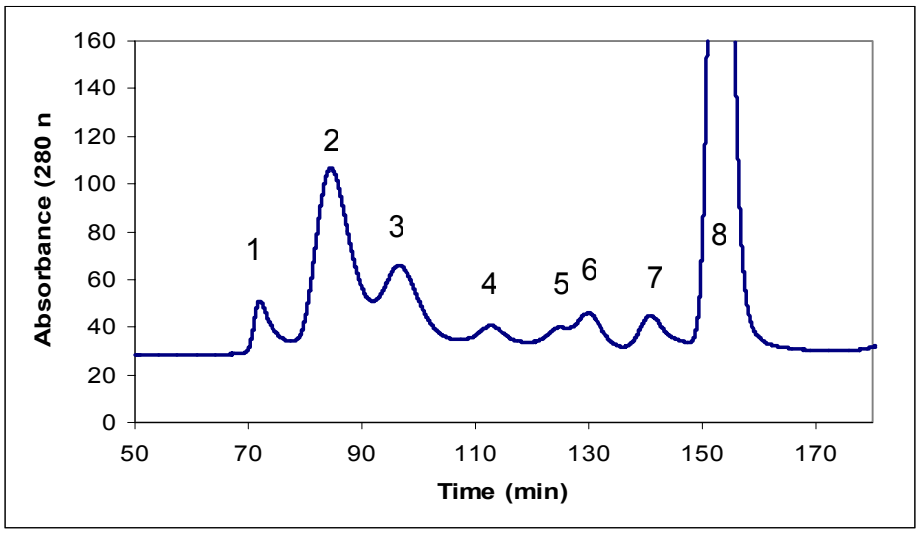

Figure 1. A typical elution profile of IEP crude ovomucin obtained by Superose 6 HR GFC. Peak 1 = β-ovomucin, peak 2 = α2-ovomucin, peak 3 = α1-ovomucin, peak 4 = ovotransferrin, peak 5 = unidentified protein (globulins?), peak 6 = ovalbumin, peak 7 = ovomucoid, and peak 8 = lysozyme.

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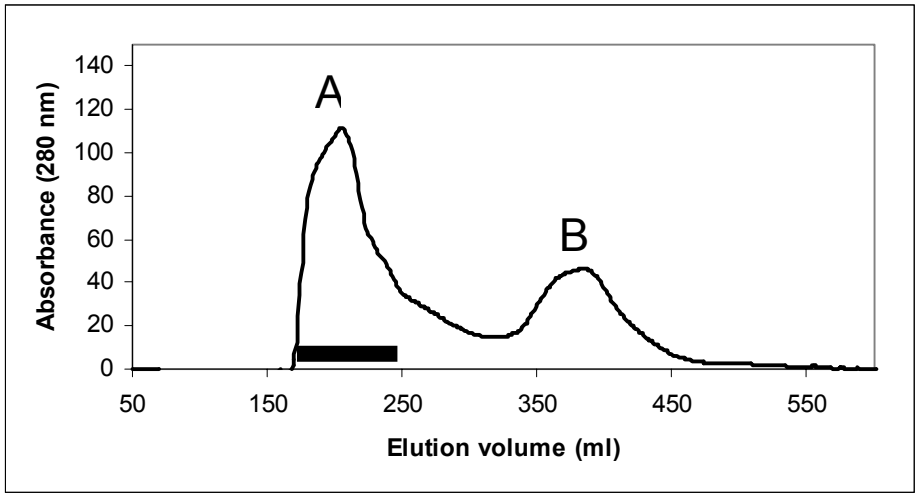

Figure 2. Elution profile of membrane filtration concentrated ovomucin sample obtained by Sephacryl S-500 HR GFC. Peak A = ovomucin, and peak B = other egg white proteins. Thick line = collected fractions.

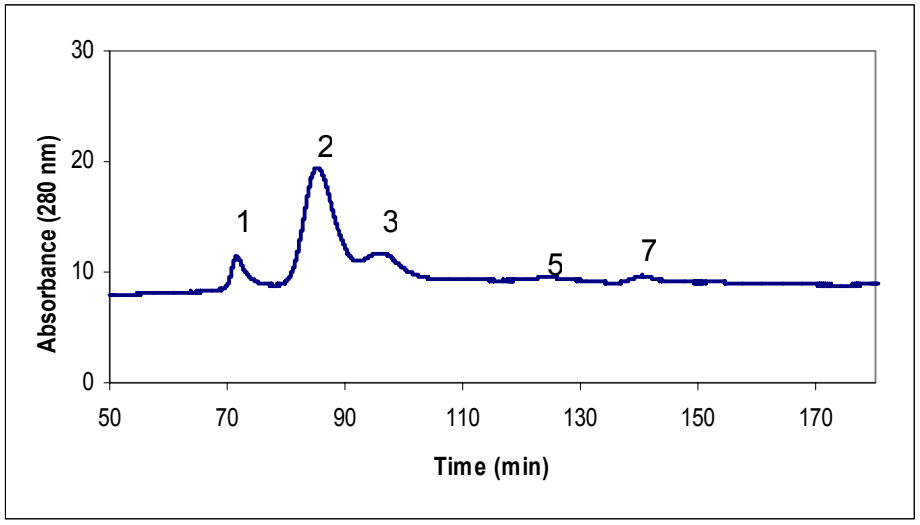

Figure 3. Elution profile of peak A obtained by Superose 6 HR 10/30 GFC. Peak 1 = β-ovomucin, peak 2 = α2-ovomucin, peak 3 = α1-ovomucin, peak 5 = unidentified protein (globulins?), and peak 7 = ovomucoid.

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PREPARATION OF HIGHLY-PURIFIED HEN EGG WHITE FLAVOPROTEIN BY FRONTAL CHROMATOGRAPHY

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Keywords: flavoprotein, purification, frontal chromatography, riboflavin protection

Abstract

In this study, a simple rapid procedure for preparation of highly-purified flavoprotein from hen egg white is presented. Flavoprotein presents a potential interest in food industries relating to the increase light- and pH-stability of riboflavin included in the riboflavin-protein complex compared to free riboflavin. The procedure uses the principle of frontal chromatography and takes advantage of the acid properties of flavoprotein. Being the most acid protein in egg white, flavoprotein has also the highest affinity for an anion exchanger and drives other egg white proteins from the column when competitive conditions for resin sites is applied. Optimising the resin capacity, a quantity of 1.17 g of flavoprotein was extracted by using 40 mL of Q-Sepharose fast flow exchanger. Flavoprotein purity rate reached 98%.

Résumé

Dans cette étude nous présentons une procédure simple et rapide pour l'extraction de la flavoprotéine à partir du blanc d'œuf de poule. Cette protéine présente un intérêt potentiel pour les industriels de la filière agroalimentaire car elle possède la particularité de stabiliser la riboflavine vis à vis des rayonnements lumineux et des pH alcalins. La procédure de purification proposée par chromatographie par déplacement exploite le fait que la flavoprotéine soit la protéine la plus acide parmi les protéines du blanc d'œuf et par conséquent la plus affine pour les sites d'une résine anionique. En mobilisant l'ensemble des sites potentiels de la résine, 1.17 g de flavoprotéine ont été extrait par 40 mL de résine Q-Sepharose fast flow. La pureté de la flavoprotéine a été estimée à 98%.

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Introduction

Hen egg white is widely used as food ingredients for the unique gelling, foaming or emulsifying properties of its proteins. Moreover egg white encloses many biologically-active proteins regarding their antigenic properties, antimicrobial activities, vitamin-binding properties, metal-binding properties,… (Nau et al., 2002). Most of them are still under-utilised in food or non-food applications because of the lack of knowledge on protein structure-function relationship or the lack of economically viable procedure for their extraction compared to alternative ingredients. Egg white riboflavin binding protein commonly called flavoprotein is one such protein.

Flavoprotein is a globular monomeric phosphoglycoprotein of 219 amino acids and 33 kDa tightly packed by the presence of 9 disulfide bonds (Monaco, 1997). It is an acidic protein with an isoelectric point of 4.0 that binds 1 mol of riboflavin ($Kd \approx 1.3 \times 10^{-9}$ M) (Stevens , 1996) at pH above 4.0, giving the egg white a definite yellow color. The riboflavin-protein complex was reported to be dissociated to give apoprotein and free riboflavin below the protein isoelectric point (Rhodes et al, 1959).

Riboflavin is an important food ingredient widely used as food colorant and/or vitamin. Food riboflavin is produced by synthesis or by fermentation but its light- and pH-sensitivities require an over-proportioning in food systems. Synthetic or fermented riboflavin substitution by the natural riboflavin-protein complex in food systems could present an advantage related to the increase stability of riboflavin. However, it will be economically effective if new cheaper procedures for flavoprotein extraction are developed and/or if the riboflavin protective action of flavoprotein is combined with other biological actions related to the riboflavin-protein complex such as growth suppression of bacteria requiring riboflavin or the exploitation of the metal-binding capacity of flavoprotein. In this study, we have developed a new one-step procedure for the preparation of large quantity of highly-purified flavoprotein from hen egg white. This procedure differed from others (Rhodes et al, 1959; Rao et al, 1997) by the quantity of purified flavoprotein recovered and the simplicity and rapidity of the operations.

Material and Methods

Egg white preparation

Hen eggs (Isabrown) were purchased from a local market (10-day-old refrigerated chicken eggs). Egg white (EW) from each egg was manually separated from the yolk and pooled. Then, EW was diluted with 2 volumes of distilled water and the pH was adjusted to 6 with 1M HCl. The solution was gently stirred and kept at 2°C for 3

hours, enabling ovomucin precipitation. Ovomucin was removed by a 3 min-centrifugation at 2000g. Prior to anion exchange chromatography, the "mucin free" EW was adjusted at pH 8 with 1 M NaOH, then centrifugated at 25000g for 20 min at 4°C in order to remove insoluble material.

Preparative chromatography

Preparative chromatography was performed using 40 mL of Q-Sepharose fast flow anion exchanger (Pharmacia Biotech AB, Saclay, France) packed in a XK 50/20 column (13×5 cm i.d) (Pharmacia Biotech AB). The column was connected to a Biopilot TM system (Pharmacia Biotech AB) equipped with 280-nm UV, conductivity and pH detectors.

"Mucin free" EW was applied to the column until the anion exchanger was saturated by flavoprotein. Then, the column was washed with 0.14 M NaCl and flavoprotein was recovered by using a linear elution program from 0.14 M to 0.5 M NaCl. All steps were carried out at a flow rate of 30 $mL.min^{-1}$.

Analytical chromatography

Reverse-phase (RP) chromatography was performed on a Vydac C_4 214TP (5×0.21 cm i.d) column (Touzart et Matignon, Vitry ^{s}/seine). HPLC grade acetonitrile (ACN) (Carlo Erba, Nanterre, France) containing 0.025% trifluoroacetic acid was used as eluent under linear gradient elution conditions. The linear gradient increased from 7% to 70% ACN in 25 min, at a flow rate of 0.8 mL min^{-1}. Detection was carried out at 280 nm.

Results and discussion

Flavoprotein is the highest acid protein in EW conferring the flavoprotein the strongest affinity for anion exchangers. This particularity was used to purify flavoprotein from EW by frontal chromatography using an anion exchanger. In frontal chromatography, a competitive binding is created between EW proteins for the resin sites during the saturation loading of the column. The component with the highest affinity for the exchanger (flavoprotein in EW when using an anion exchanger) displaces the other proteins which are excluded from the column.

A volume of 5.58 L of "mucin free" EW, containing about 1.5 g of flavoprotein calculated using 0.8 g L^{-1} of flavoprotein in EW (Li-chan and Nakaï, 1989), was loaded onto the Q-sepharose FF column. The column became progressively yellow due to the adsorption of the flavoprotein whereas the fraction excluded from the column was initially colourless. Before the end of the saturation loading the excluded fraction showed a slight yellowish colour relating the exclusion of flavoprotein molecules. These latter could be excluded from the column by EW proteins of lower affinity than flavoprotein for the anion exchanger but in high proportion in EW such as

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ovomucoïd (11% of the total EW protein content; pI = 4.1) and ovalbumin (55% of the total protein content; pI = 4.5). However, the saturation loading was maintained until having a complete and homogenous yellow anion exchange chromatography column. At this stage, an equilibrium existed in the column between the non-bound material and the bound material. In other words, the composition of the fraction coming in the column was equivalent to the composition of the fraction coming out the column.

As soon as the 5.58 L of "mucin free" EW loaded onto the column, non bound material was removed by washing the column with 0.14 M NaCl until the absorbance reached the baseline. Bound material was eluted using a linear gradient from 0.14 M to 0.5 M NaCl (figure 1). Elution profile showed two peaks; the second one corresponded to the flavoprotein fraction and was characterised by an intense yellow coloration. Then, this fraction was extensively dialysed against water and freeze-dried before further analysis.

A quantity of 1.17 g of flavoprotein was recovered by this procedure which represented 79 % of the flavoprotein in 5.58 L of "mucin free" EW. The Q-sepharose FF capacity for flavoprotein extraction from "mucin free" EW" was 30 g of flavoprotein per litre of resin. Flavoprotein was checked for purity rate by RP-HPLC. RP-HPLC profile showed one major chromatographic peak which area represent 98% of the total area (figure 2). The chromatographic peak eluted with a retention time of 2 min belonged to riboflavin as it dissociated from the riboflavin-protein complex at acidic pH (condition used in RP-HPLC).

Figure 1:

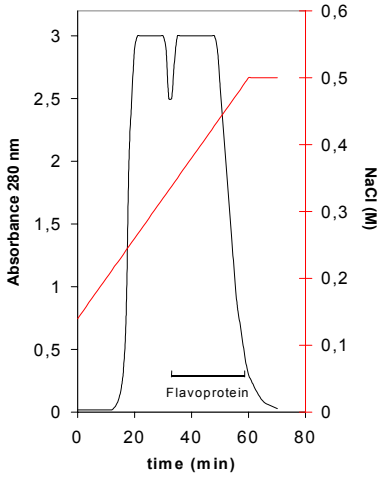

Figure 2:

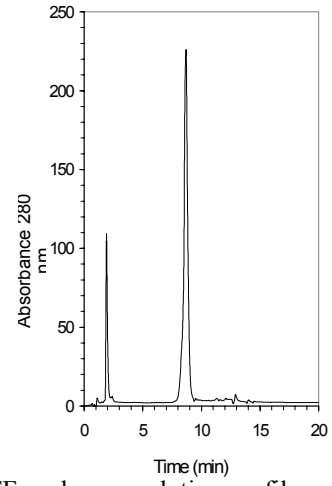

Figure 1: frontal chromatography on Q-sepharose FF exchanger: elution profile

Figure 2: Reverse phase chromatography on Vydac C4 214TP of flavoprotein fraction

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CONTENTS

PRODUCTION OF A HIGHLY PURIFIED PHOSVITIN FROM EGG YOLK AND STUDY OF ITS IRON BINDING CAPACITY

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Keywords: egg yolk, phosvitin, extraction, chromatography, iron-binding

Abstract

Phosvitin, a highly phosphorylated egg yolk protein, has an exceptional amino acid composition (50% of phosphoserine). Therefore, phosvitin has very good metal binding properties and so, could be an excellent natural anti-oxidant ingredient.
Phosvitin purification methods described in the literature are generally laborious and time consuming. We have developed a method based on the precipitation of phosvitin by addition of $MgSO_4$ salt, followed by a further purification by ion exchange chromatography. This two step procedure enables the production of highly purified, metal-free phosvitin (purity rate > 98%) with good extraction yield (~ 85%). Purified phosvitin can bind ~ 70 moles of iron (Fe^{3+}) by mole of protein in determined optimal conditions (pH 6.5 and ionic strength 0.15M) .

Résumé

La phosvitine, une protéine très phosphorylée du jaune d'œuf, possède une composition en acides aminés exceptionnelle (50 % de phosphosérines). Ainsi, la phosvitine a d'excellentes propriétés d'interaction avec les ions métalliques et peut donc être un très bon anti-oxydant naturel. Les procédures d'extraction de la phosvitine citées dans la littérature sont des techniques souvent longues et laborieuses. Nous avons développé une méthode d'extraction basée sur une précipitation au sulfate de magnésium, suivie d'une purification par chromatographie d'échange d'ions. Ce procédé en deux étapes nous permet de

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produire facilement une protéine pure à 98% avec un rendement de 85%. Cette protéine ainsi produite peut fixer jusqu'à 70 moles de fer par mole de protéine, dans les conditions optimales que nous avons déterminées (pH 6.5 et force ionique 0.15M).

Introduction

Phosvitin which represents 2% of egg yolk dry matter (Mecham and Olcott, 1979) is known like one of the more phosphorylated proteins. Fifty % of its amino acids are serine, out of which 90% are phosphorylated (Losso et al., 1994). Moreover, the phosphoserines form clusters than carry up to 15 consecutively residues (Byrne et al., 1984; Mabuchi et al., 1996). This singular structure makes of this protein one of the most stronger metal chelating agent (Hegenauer et al., 1979; Grizzuti and Perlmann, 1973 and 1975). In relation to this property, phosvitin could be an excellent anti-oxydant ingredient. But phosvitin iron binding capacity is depending on physico-chemical conditions and also on extraction method used. Actually, these conditions could influence protein structure and so, its properties.
So, it was interesting to develop easy and non-denaturating phosvitin extraction method. Some authors have developed methods (Mecham and Olcott, 1979; Losso et al., 1994; Joubert and Cook, 1958; Sundararajan et al.; 1960, Wallace and Morgan, 1986), but they are laborious and time consuming, or usually make use of non aqueous or non food grade solvents to separate proteins from lipids. The authors have also shown that phosvitin was composed of several main sub-component with molecular weights from 27 000 to 45 000 Daltons, called α and β phosvitin and phosvettes. Composition of phosvitin is depending on extraction methods used.
So, we proposed in this work the optimisation extraction process and the study of phosvitin iron binding capacity.

Material and Methods

Material

Isabrown eggs were obtained from local distributor, they were three or four days old. Commercial phosvitin was purchased from Sigma (Saint Quentin-Fallavier, France).

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Phosvitin precipitation

Fresh hen eggs was manually broken and yolk were carefully separated from white. Yolk was collected and granules were extracted according to the method of Mc Bee and Cotterill (1979). They were dissolved in 1.74M NaCl solution (10% p/v). After dissolution at pH 7.25, solution was dialysed at 4°C. Supernatant was diluted with 0.9M $MgSO_4$ solution to obtain a 0.2M Mg^{2+} final concentration where phosvitin precipitates. After centrifugation (10000g for 30 min), a precipitate was collected and freeze-dried (Pvti).

Ion exchange chromatography

Chromatography was carried out on a FPLC system, equipped with an automatic injector, dual monitors at 220 and 280 nm containing 0.2 mL flow cells, and a fraction collector. Anion exchange gel, a SOURCE 15Q from Amersham Pharmacia Biotech AB (Upsala, Sweden) was used. Pvt_i was dissolved (5 mg/mL) at pH 5.0 (0.05 M, NaAc/AcH) in the presence of 10 mM Na_2EDTA. At this pH, phosvitin is more soluble than HDL, major contaminant. EDTA was added to weak metallic bridges between these proteins. Five mL of this solution were injected in the column that was previously equilibrated with the same buffer system without Na_2EDTA. The elution was performed with an increasing gradient of NaCl.

Characterization

Nitrogen : The nitrogen content of phosvitin samples was determined by an automated total protein nitrogen method (Mangavel et al., 2001). Total protein content was calculated with conversion factor 6.25. **Phosphorus :** The phosphorus content of different samples was determined using the colorimetric method described by Bartlett (1959) using hydrazine sulphate and sodium molybdate. **Fe and Mg content :** Fe and Mg content were measured by atomic absorption spectrometry with a spectrometer SpectrAA 55B (Varian Pty. Ltda., Mulgrave Victoria, Australia).

Native PAGE

Protein samples and eluted fractions (after desalting by dialysis against distilled-deionized water) were dissolved in the sample buffer (0.05 M Tris-HCl pH 6.8, 0.04% bromophenol blue and 30% glycerol).

Stacking gel was constituted by 3.5% of acrylamide in 0.124 M Tris-HCl buffer at pH 6.8, and the running gel by 6.5% of acrylamide in 0.05 M Tris-HCl/0.36 M glycine at pH 8.8. Electrophoresis buffer was constituted by 0.05 M Tris-HCl, 0.42 M glycine at pH 8.8. About 30 µg of total protein were loaded onto the gels which were stained with Stains all, specific dye solution for acidic proteins (Wallace and Begovac, 1985). Electrophoretic migration was performed at 30 mA per gel for 1.5 h in an Amersham Biosciences system (Hoefer, Mighty Small II SE250/SE260, San Francisco, U.S.A.).

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Iron binding capacity

Free iron content was determined according to Sandell (1950), as reported by Grogan and Taborsky (1986). The method is based on the colorimetric determination of ferrous ions/phenanthroline complex which absorbs at 508 nm.

Iron binding capacity was studied according to the method described by Guérin-Dubiard et al. (2002).

To study the influence of pH and ionic strength on iron binding capacity of phosvitin, we have used central composite model design. Limits of validity model were : 3.5 to 6.5 for the pH and 0.15 to 0.55M for ionic strength. The different buffers used were glycine, acetic/sodium acetate and Tris-HCl.

Results and discussion

Phosvitin extraction and purification

Crude phosvitine obtained by precipitation (Pvti) was purified by anion exchange chromatography. Elution was performed by NaCl gradient. Four fractions were eluted (A to D) (data not shown). Optical density at 220 and 280 nm of first peak (fraction A) were similar in opposition of the other peaks. Moreover, not many protein material was detected by native PAGE. Otherwise, we have measured high metal concentration (16.7 mg/L of Fe and 270 mg/L of Mg), that suggests presence of metal/EDTA complex. The elution of peak B was at low salt concentration (0.15 M NaCl), peak C was eluted at 0.39 M, and peak D between 0.42 and 0.55 M. The phosvitin material distribution was 13%, 6% and 80% in peaks B, C and D respectively, based on an integration of the areas under the 220 nm trace. Figure 1 shows native electrophoresis profile of chromatographic fractions. Lane J represents yolk profile, with all its constituents (LDL,HDL, livetin, phosvitin and phosvettes). Lane Pvti and $\sum$ represent, respectively, crude phosvitin, before chromatography and commercial phosvitin (Sigma). These two proteins are contaminated by LDL and HDL. Lanes B, C and D are chromatographic fractions. Fraction B presents beta-phosvitin polypeptides (99%), fraction C is enriched in beta-phosvitin and phosvettes, and fraction D is composed mostly by alpha and beta-phosvitin. Even if composition of the different chromatographic fractions were not exactly the same, our objective was to have a purified phosvitin with a good yield more than to obtain its different polypeptides separately. So, fractions from peaks B, C and D were mixed, dialysed, lyophilised and stocked (purified phosvitine, Pvt_p). Therefore, we can obtain lyophilised phosvitin with a yield of 1.7 g of Pvt_p/100 g of dried egg yolk , that is to say a yield of 85%, with a high purity rate (98%).

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Atomic ratio (N/P) was 2.63 ± 0.07 for Pvtp similar to those described by different authors (Mecham and Olcott, 1979; Joubert and Cook, 1958). Metallic composition of Pvti is 1.2 Fe atoms and 37 Mg atoms per molecule of protein while Pvtp contains only 0.03 and 0.016 atoms of Fe and Mg respectively.

Iron binding capacity

Figure 2 shows the estimated response surface of fitting a general linear statistical model relating iron fixation by phosvitin to pH and ionic strength values. The analytical results indicate a statistically significant relationship at 99% confidence level. There is a relationship between the iron binding capacity of phosvitin and both the pH and ionic strength conditions. The greatest capacity is obtained in a zone of higher pH and lower ionic strength values (nearby 6.5 and 0.15 respectively). In these conditions, 1 mg of phosvitin can bind 115µg of iron. We have used a Mickaëlien model (figure 3) , and we have determined the number of fixation sites and affinity constant. They are respectively 70 and $3.14x10^5$ M^{-1}.

This high affinity is the one in optimal conditions studied, but it could change with other parameters, like temperature or mechanical treatments (emulsion, high pressure). Nonetheless, we have already made some assays, and phosvitin seems to keep its excellent binding properties with technological treatment (temperature and emulsification). Phosvitin could be a good natural anti-oxidant ingredient.

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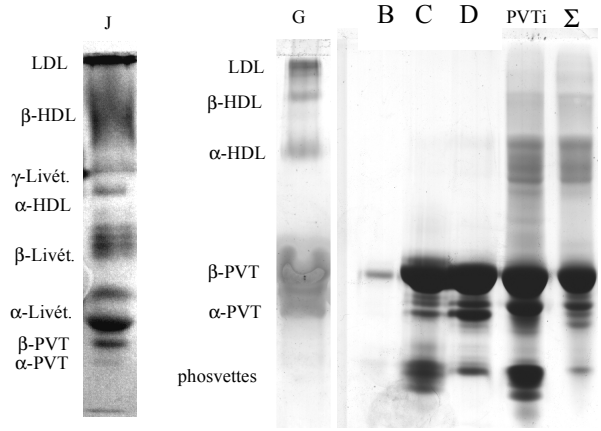

Figure 1 : Native polyacrylamide gel electrophoresis (6.5% acrylamide) of different fractions. J: Egg yolk, G: Granules, (B,C and D) chromatographic fractions, Pvt_i: crude phosvitin, □□Sigma phosvitin,. Stain: Stain all

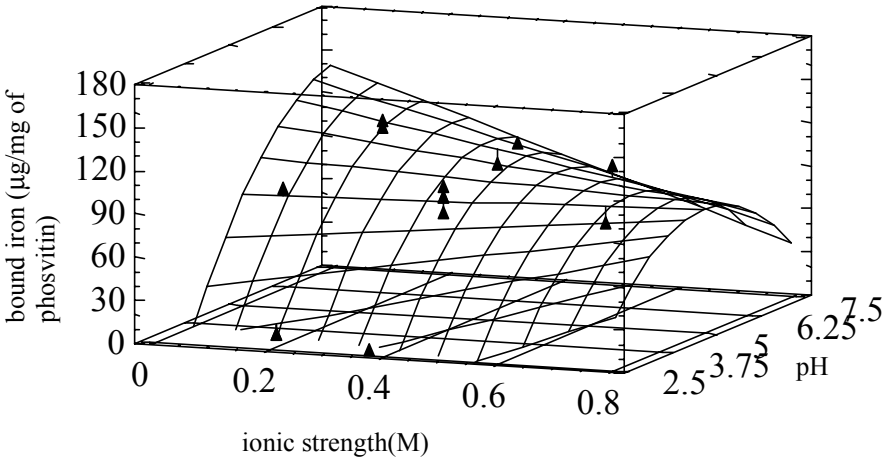

Figure 2 : Estimated response surface for the iron fixation by phosvitin. Limits of validity model: 3.5 to 6.5 for the pH and 0.15 to 0.55 for the ionic strength.
▲ : experimental points.

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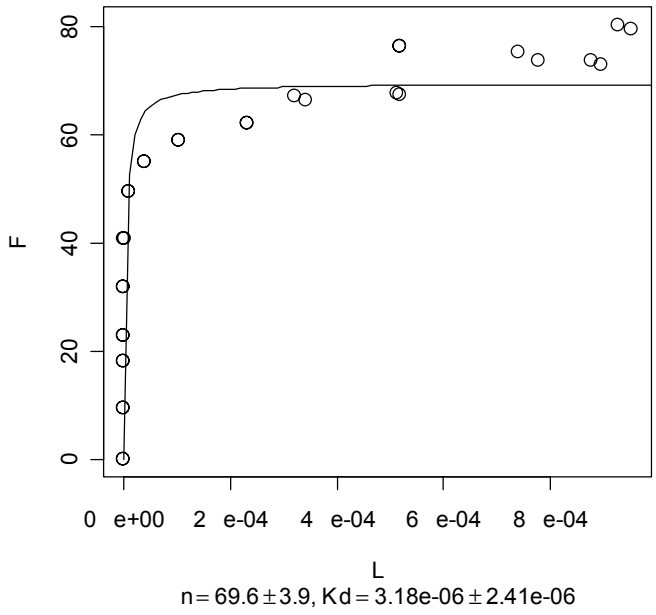

Figure 3 : Phosvitin iron binding capacity at pH 6.5 and 0.15M of ionic strength. Fitting according to Mickaëlien model
L = free iron (mole) , F = bound iron (mole/mol Pvtp)
n = number of binding site and Kd = dissociation constant
Kd = 3.18 x10^{-6} M, so $K_{affinity}$ = 3.14 x10^{5} M^{-1}

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KEYWORDS

This classification is non exhaustive as it is based on key words provided by the authors and do not included those present in the abstract or text

EGG PRODUCTION AND TRADE PRESENT AND PERSPECTIVES

E. D. Gillin and N. Sakofl

Chief Basic Data Branch, FAO, Rome, Italy

Keywords: Egg, Poultry, Production, Statistics and Chicken.

Click on the author's name to go to the authors index

Click on a keyword to go to the keywords index.

Abstract

From the early 1960s to 2000-2002 world egg
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This is due to the changes that have taken place over the last 25 years in many countries, particularly with regard to the growth of a modern specialized and intensive sector alongside the traditional sector.

world production, against 25 %in the early

y 1980s hen eggs production per laying hen

early 1980s to 2000-2002, 24.7 % from 8.1 to

Résumé

À partir du début des années 60 jusqu'en 2000-2002, la production mondiale d'œufs a augmenté de 268% : 898% dans les pays en voie de développement et de presque 61% pour les pays développés. La production des pays en voie de développement représente 67% de la production mondiale, contre 25% au début des années 60. Au niveau mondial, à partir du début des années 60 jusqu'au début des années 80, la production d'œufs de poule par poule couveuse a augmentée de 12,5% de 7,2 Kg à 8,1 Kg. À partir du début des années 80 jusqu'aux années 2001-2002 de 8,1 Kg à 10,1 Kg. Ceci est dû aux changements qui se sont effectués ces dernières 25 années, dans beaucoup de pays, en particulier en ce qui concerne la croissance d'un secteur moderne, spécialisé et intensif à côté d'un secteur traditionnel.