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26th World's Poultry Congress

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presented in Paris 7-11 August 2022

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Dr Michèle TIXIER-BOICHARD, chair of 26th WPC
Dr Michel DUCLOS, Chairman of the Scientific Committee of 26th WPC

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Welcome Page

Dear participants to the 26th WPC, dear readers,

In the context of the COVID-19 pandemics, which emerged in 2020 and is still present, the highest priority of the French Branch of WPSA and the organizing committee of the 26th World's Poultry Congress has been to organise a successful congress, avoiding health hazards and welcoming participants from all continents. As a consequence, the event planned in 2020 had to be postponed to 2021 and again to 2022. We are grateful to the invited speakers who maintained their contribution despite this complex background.
These proceedings gather all summaries and/or full texts corresponding to the invited lectures presented in Paris 7-11 August 2022.

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PLENARY SESSIONS

PS1

Economy and sustainability of the Poultry Production Chain

Global challenges for the economy of the poultry sector (P. Van Horne)

Peter VAN HORNE

Wageningen Economic Research,
PO Box 8130 6700EW WAGENINGEN The Netherlands
peter.vanhorne@wur.nl

Global poultry meat and egg production showed an impressive increase over the last 15 years. Poultry meat production was 120 mln ton in 2017 compared to 73 mln ton in 2002 (+65%). Egg production was 80 mln ton in 2017 compared to 54 mln ton in 2002 (+50%). In this period North America and Europe lost market share and Asia and South America increased their share. Largest producers in poultry meat are USA (18% of global production), Brazil (12%) and China (12%). In egg production China is by far the largest producer (38% of global production). In 2017, poultry meat surpassed the production volume of pig meat.

Projections for 2028 show a further increase in eggs and poultry meat. The outlook for 2028 shows a higher annual growth for poultry meat compared to pig meat and beef. As a result of population growth and increasing income of a growing middle class in many developing countries meat consumption will further grow with poultry meat often being the favored meat. Tradition, no religious barriers, a low price and convenient preparation are factors in favor of poultry meat.

For both layers (battery cages) and broilers (fast growing breeds) the most efficient production system is dominating world-wide. Housing of layers is criticized in some parts of the world for animal welfare reasons. In terms of sustainability animal welfare is just one aspect to be considered. Social aspects as well as environmental and economic factors need to be included as well. Based on several indicators to quantify sustainability a comparison was made for cage, barn, free range and organic egg production. Cage production had the highest score on the environmental dimension, whereas free range production gave the highest score in the social dimension. In the economic dimension both enriched cages had the highest sustainability score. However, using non-equal weights for the indicators resulted in different score. The method gives policy makers and stakeholders a tool to quantify sustainability and provides a weighted decision regarding future housing systems for layers.

Kew words: poultry production, economics, sustainability, housing systems

Reducing Environmental Impact of Poultry Production (E. Kebreab)

Ermias KEBREAB

Department of Animal Science, University of California, Davis, Davis 95616, U.S.A.

ekebreab@ucdavis.edu

SUMMARY

Livestock and poultry production have an impact on the environment through resource utilization, greenhouse gas emissions and nutrient loading in the environment. Poultry meat makes up the majority of growth in meat production, particularly in low and middle-income countries. As the demand for poultry products grow substantially, even more pressure will be put on the environment. In order to assess the environmental impact of poultry production, factors that contribute to the whole production from cradle to farm gate or cradle to grave should be considered. For example, poultry production will require estimates of emissions from preparation of feed (i.e., feed production, transport, and milling), animal husbandry (e.g. energy for hatching, lighting, cooling, ventilation, and feed distribution) and manure management. The basic system boundary is also affected by feed conversion ratio and may involve more emissions due to land use change (e.g. soybean use in Europe from Latin America). Local production systems will vary greatly and affect emission estimates. For example, in areas where specialty feed ingredients are used, feed conversion ratio is improved, which is reflected in lower emission intensity (i.e. emissions per unit of product). The Livestock Environmental Assessment Partnership of FAO recommends that several environmental impact categories should be assessed including global warming, acidification, and eutrophication potentials as well as primary energy demand. Within this framework several mitigation strategies can be assessed. Reduction of environmental impact can be achieved through nutrition, manure and farm management techniques. For example, using crystalline amino acids, phytases and other enzymes lead to significant reductions in several environmental assessment categories. Farm management should include tackling local sources of emissions within a life-cycle assessment approach. This will allow for a fuller understanding of poultry production's impact on the environment and its mitigation globally.

Introduction

The focus of this paper is on poultry production in intensive systems and mitigation options to reduce its impact on the environment. As a consequence of rising population, particularly in low income countries, and increase in disposable income, livestock and poultry production is expected to double by 2050 (Garnett, 2009). In poultry production, the impact on the environment is mainly from 1) excretion of excess nitrogen and phosphorus, leading to the deterioration of aquatic systems (Conley et al., 2009); 2) indirect emissions through feed production and direct greenhouse gas (GHG) emissions from manure storage and application to the field, and 3) ammonia emissions responsible for acidification and eutrophication of N-limited ecosystems (Sutton et al., 2008).

Several environmental impact categories should be assessed including global warming, acidification, and eutrophication potentials as well as primary energy demand in order to gain a better understand from the whole system. This paper aims to discuss mitigation options to reduce environmental impact of poultry by considering various components during the production of poultry products.

Nutrient and Mineral Excretion

Nitrogen excretion. One of the main sources of excess N excretion to the environment is using regular feedstuffs to meet amino acid (AA) requirements, in particular, essential AA, which means greater amount of total protein is consumed by the animal than required. Reducing intake of protein is the most effective way to reduce environmental impacts, however, this has to be achieved without impairing animal performance or negative environmental impact. Utilization of supplemental AA to meet protein requirements can reduce N excretion by 8% for every 1%-unit reduction in dietary protein intake (Kerr, 2003).

Mineral excretion. Surplus minerals in excreta present an environmental pollution problem to intensive livestock operations. In poultry, excretion of phosphorus (P) in manure is of special concern. Poultry excrete large amount of P because less than 40% of P consumed may be utilized depending on P availability, efficiency of feed conversion and the amount of P consumed in excess of the animal’s requirement (Vitti and Kebreab, 2008). Options to reduce excess P excretion from poultry can be achieved through either optimizing P availability in feed or increasing efficiency of livestock through increased P incorporation in eggs or faster growth (Kebreab et al., 2012). The amount of P in diet can be reduced by matching animal P requirement closely with available P in the diet. Various mathematical simulation models are available that can evaluate feeding strategies aimed at reducing excretion of potential pollutants, including P in poultry manure to the environment (France et al., 2013). Feeding birds in groups according to their physiological state reduces P excretion substantially. An evaluation of the phase feeding concept in growing pigs has shown a 20% reduction in P consumption without hampering growth performance (Kebreab et al., 2011). Phosphorus availability can be enhanced using phytase, which is a digestive enzyme catalyzing the release of P from phytate complex, reducing the need to supplement with inorganic P sources. Bouguin et al. (2014) reported that broilers supplemented with phytase at 1039 FTU/kg of diet increased P retention by 8.6 percentage units on average. The authors further reported that a unit increase of phytase dose and Ca:P from their means further increased P retention.

Biotechnology to reduce N and P excretion. Transgenics have been used to develop low-phytate plants such as high P available corn. Kebreab et al. (2005) evaluated the environmental impact of using high-oil corn. The authors reported a potential reduction of P excretion of 4.52 MT/year in Brazil because it decreased the need to use inorganic P sources such as dicalcium phosphate. Animal breeding to increase feed efficiency also plays a role in reducing P excretion. For example, Mignon-Grasteau et al. (2010) showed that birds selected for high apparent ME corrected for 0 N retention (AMEn) excreted 48.4% and 36.7% less N and P, respectively, than control without affecting BW. Similarly, de Verdal et al. (2013) showed that over the whole rearing period, high AMEn birds excreted significantly less fresh and dry excreta (−56 and −61%) than low AMEn birds and reference line birds (−6 and −26%). The authors also observed that N and P excretion rates of high AMEn birds were 13 to 30% less than those of low AMEn birds and 12 to 19% less than reference line birds, depending on age.

Greenhouse Gas Emissions

Indirect emissions. Poultry production indirectly contributes to GHG emissions through the need to produce feed. Kebreab et al. (2016) reported that feed production contributed to over 50% of greenhouse gas emissions in poultry. The use of synthetic AA in poultry diets led to a substantial reduction in global warming potential. When emissions from land use change were considered, the global warming potential increased by up to 4.7 times in Europe and 3.9 times in South America’s broiler production. Using synthetic AA can reduce total global warming potential in the European production system by 52% and by 80% in South America because fewer soybean products are required. Soybean production has been associated with more recent (within 30 yr) deforestation (Macedo et al., 2012). Therefore, land use change emissions from soybean products from South America considerably change the relative contributions to GWP in both livestock categories (for the European and South American regions). Additionally, transport of the poultry products contributes to global warming (Gerber et al., 2021).

Direct emissions. Poultry excretion, manure storage, and field application leads to GHG emissions such as methane, nitrous oxide. The majority of the methane in poultry production is emitted during manure storage. Several factors affect methane and nitrous oxide emissions from manure, including temperature, moisture content, and oxygen. The following mitigation strategies can help to reduce GHG (Dunkley, 2021): 1. Handling manure as a solid or spread it on land so it decomposes aerobically and produces little or no methane, 2. Avoiding prolonged litter storage, which can increase methane emissions. 3. Ensuring manure heaps are covered to keep them dry, and 4. Adding high carbon substrate to manure heaps.

Ammonia Emissions

Kebreab et al. (2016) investigated the environmental impact of using synthetic AA in poultry production through a life cycle assessment in 4 impact categories including global warming potential, eutrophication potential, acidification potential and primary energy demand. Ammonia emissions to air and nitrate and phosphate emissions to water were predominant drivers for eutrophication. De Vries et al. (2015) suggested that mitigation actions such as segregation of urine and feces inside housing, addition of zeolite to solid manure, and sealed storage in integrated manure management systems reduced ammonia and methane emissions. Kebreab et al. (2016) reported that the eutrophication potential of synthetic AA unsupplemented diets was reduced by up to 49% in broiler production systems compared to AA supplemented alternatives. To mitigate acidification potential from broiler manure, reducing time of exposure on the surface of the ground is the most effective strategy which decreases ammonia emissions (Ndegwa et al., 2008). Peters et al. (2011) suggested that reducing the leaching of soil N coming from manure might be the best way to balance the N budget without causing acidification. The acidification potential of synthetic AA supplemented strategies was reduced by up to 79% in broiler production systems (Kebreab et al., 2016).

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PS2

One health - One Welfare

High Pathogenicity Avian Influenza in a One Health Framework (D. Swayne)

David Swayne

U.S. National Poultry Research Center, Agricultural Research Service, U.S. Department of Agriculture, 934 College Station Road, Athens, Georgia, USA

Correspondence: David.Swayne@usda.gov

Abbreviated Title: HPAI in One Health Framework

Summary

Our knowledge of the ecology and epidemiology of avian influenza has changed over the past 150 years. Initially, avian influenza was identified as a severe disease of chickens termed fowl plague with the first cases reported in 1878. However, in 1961, infections and deaths in wild birds (common terns) were identified. In 1959, the first case of avian influenza virus infection was observed in a human. There are two broad categories of phenotypic expression of avian influenza viruses in chickens: low pathogenicity avian influenza (LPAI) and high pathogenicity avian influenza (HPAI). Since 1959, there have been 44 genetically distinct HPAI epizootics. The largest outbreak has been caused by H5Nx Gs/GD lineage of HPAI virus which began in 1996 and has evolved into multiple genetic clades of virus. Over the past two decades, Gs/GD lineage of HPAIV infections has evolved phenotypically in wild birds from resistance to infection to some viruses causing severe disease and mortality. Over 864 cases of infection of the Gs/GD HPAIV lineage in humans have been reported and of those cases, 456 fatalities have occurred. Beginning in 2005, intercontinental spread from Asia has occurred with large outbreaks of Gs/GD lineage HPAI in poultry of North America, Africa, Middle Eastern and European Union. Historically, Gs/GD HPAI cases were initially H5N1, but assortment of the gene segments with other HPAI or LPAI viruses has produced H5N2, H5N3, H5N5, H5N6 and H5N8 HPAI viruses. Since 2014, the 2.3.4.4 clade viruses have diverged into eight genetic subgroups: a-h with major outbreaks of 2.3.4.4b occurring across Asia, Europe, and Middle East in late 2020 and early 2021. Since 2020, most of the 2.3.4.4b viruses have been H5N1, H5N8 and H5N6 with H5N1 spread intercontinentally between Asia, Europe, Africa, and North America in late 2020 through 2021. The current outbreaks include migratory aquatic birds, raptors, backyard and village poultry and commercial poultry, and a few human infections.

Key words: Avian influenza, birds, ecology, epidemiology, one health, poultry, public health

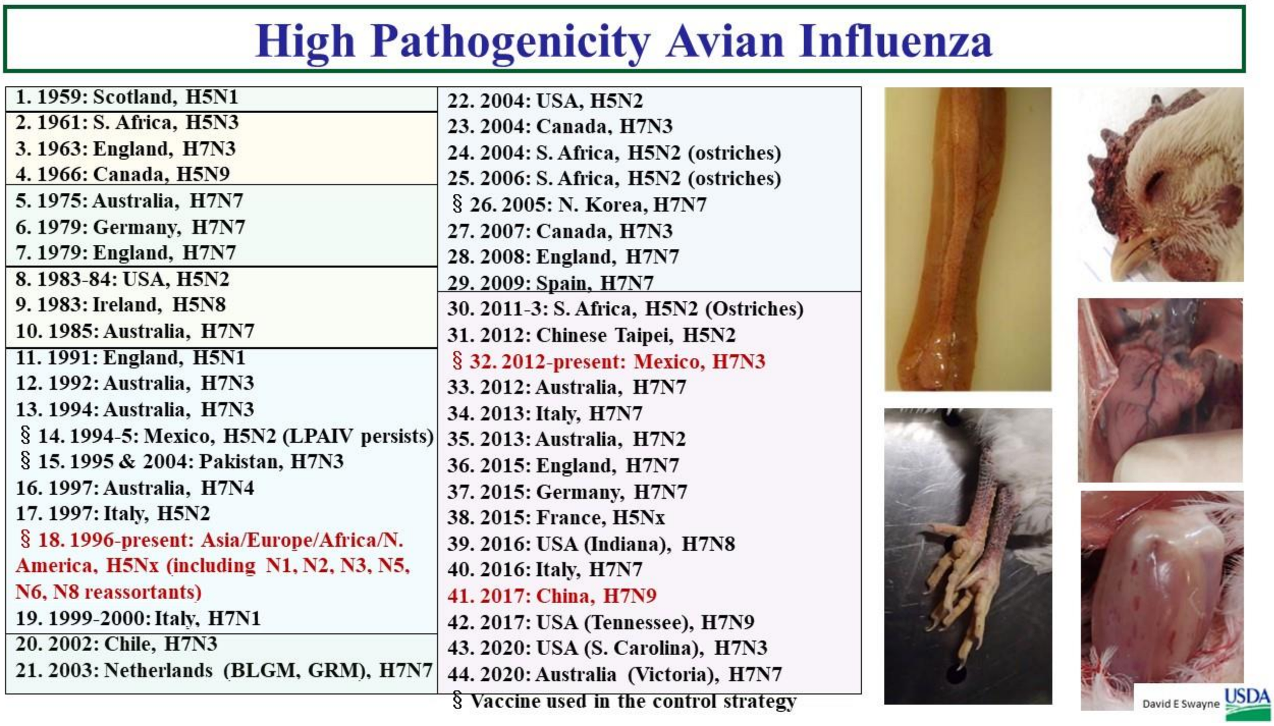
Introduction

Avian influenza is an infection or disease caused by Type A influenza viruses and it is in the virus family *Orthomyxoviridae* (Swayne et al., 2020). Avian influenza viruses are classified into subtypes based on the two surface proteins: the hemagglutinin (H1-16) and neuraminidase (N1-9). There are two broad categories of phenotypic expression of avian influenzaviruses based on experimental inoculation in chickens: low pathogenicity avian influenza (LPAI) and high pathogenicity avian influenza (HPAI). The LPAI viruses cause asymptomatic infections to mild disease, primarily evident as respiratory disease, diarrhea and drops in egg production while the HPAI viruses cause severe systemic disease affecting many internal organs, the vascular system, and the brain, and cause high mortality rates. Although the LPAI and HPAI phenotypes are defined by infection in chickens, similar outcomes are seen with other gallinaceous poultry species (i.e. birds in the order Galliformes).

Our knowledge of the ecology and epidemiology of avian influenza has changed over the past 150 years. Avian influenza virus infections not only can have a negative impact on poultry health and their welfare, but some avian influenza viruses have caused infections, illness and fatalities in humans, and have had a negative effect on the environment, especially some HPAI viruses causing disease and mortality in diverse species of migratory aquatic birds, raptors and other birds. Thus, avian influenza fits within the One Health – One Welfare concept and solutions require collaborative interaction between veterinary (poultry), environmental and human health to solve these complex interrelated problems.

Poultry

Avian influenza was first reported as a severe, high mortality disease of chickens in Northern Italy during 1878 (Perroncito, 1878), and initially was termed fowl plague, pest or grippe (Swayne et al., 2020). By the 1930s, the disease was present across Europe, Asia, Northern Africa, North and South America based on signalment, clinical signs and host poultry species affected. In 1901, the cause of fowl plague was determined to be a small filterable agent, i.e. one of the first viruses to be identified as the cause of an animal disease (Centanni and Savonuzzi, 1901). However, it was not until 1955, when the fowl plague virus was classified as an influenza A virus of the H7 hemagglutinin subtype (Schafer, 1955). The first case of LPAI was identified in chickens in Germany during 1949 (Dinter and Bakos, 1950), and first infections of LPAI virus in domestic ducks was diagnosed in Canada during 1953 (Walker and Bannister, 1953). The first infections by the H5 subtype of HPAI were reported in Scotland on one premise of chickens during 1959 (Swayne et al., 2020). Since 1959, there have been 44 genetically distinct HPAI epizootics (Figure 1).



High Pathogenicity Avian Influenza

1. 1959: Scotland, H5N1
2. 1961: S. Africa, H5N3
3. 1963: England, H7N3
4. 1966: Canada, H5N9
5. 1975: Australia, H7N7
6. 1979: Germany, H7N7
7. 1979: England, H7N7
8. 1983-84: USA, H5N2
9. 1983: Ireland, H5N8
10. 1985: Australia, H7N7
11. 1991: England, H5N1
12. 1992: Australia, H7N3
13. 1994: Australia, H7N3
§ 14. 1994-5: Mexico, H5N2 (LPAIV persists)
§ 15. 1995 & 2004: Pakistan, H7N3
16. 1997: Australia, H7N4
17. 1997: Italy, H5N2
§ 18. 1996-present: Asia/Europe/Africa/N. America, H5Nx (including N1, N2, N3, N5, N6, N8 reassortants)
19. 1999-2000: Italy, H7N1
20. 2002: Chile, H7N3
21. 2003: Netherlands (BLGM, GRM), H7N7
22. 2004: USA, H5N2
23. 2004: Canada, H7N3
24. 2004: S. Africa, H5N2 (ostriches)
25. 2006: S. Africa, H5N2 (ostriches)
§ 26. 2005: N. Korea, H7N7
27. 2007: Canada, H7N3
28. 2008: England, H7N7
29. 2009: Spain, H7N7
30. 2011-3: S. Africa, H5N2 (Ostriches)
31. 2012: Chinese Taipei, H5N2
§ 32. 2012-present: Mexico, H7N3
33. 2012: Australia, H7N7
34. 2013: Italy, H7N7
35. 2013: Australia, H7N2
36. 2015: England, H7N7
37. 2015: Germany, H7N7
38. 2015: France, H5Nx
39. 2016: USA (Indiana), H7N8
40. 2016: Italy, H7N7
41. 2017: China, H7N9
42. 2017: USA (Tennessee), H7N9
43. 2020: USA (S. Carolina), H7N3
44. 2020: Australia (Victoria), H7N7

§ Vaccine used in the control strategy

Figure 1. Emergent (black font) and entrenched (red font) high pathogenicity avian influenza outbreaks, 1959-2022.

Migratory Aquatic Birds

The first detection of HPAI virus in wild birds was during a mortality event in South Africa during 1961. An H5N3 HPAI virus was associated with die-offs in common terns (*Sterna hirundo*) in 4 small areas, but without any reports of infections in poultry. Furthermore, in 1968, avian influenza virus infections of wild aquatic birds were identified by serologic investigation (Easterday et al., 1968) and similar finding in pelagic seabirds in Australia during 1972 (Laver and Webster, 1972). The first LPAI viruses isolated from asymptomatic migratory ducks was in 1972 as part of the USA Newcastle disease outbreak surveillance (Slemons et al., 1974).

Humans

In 1959, the first case of avian influenza virus infection was observed in a human. Additional human cases of avian influenza have been reported but these have been restricted to direct exposure to infected poultry and the cases occurred without sustained human-to-human transmission. Since 1959, 2633 human cases of avian influenza with 1085 fatalities have been reported (Swayne et al., 2020). Most of the human cases have resulted from two entrenched strains: 1) H5Nx Gs/GD lineage HPAI virus (860 cases, 454 fatalities), and 2) H7N9 Eurasian lineage LP or HPAI viruses (1625 cases, 622 fatalities) (Swayne et al., 2020). Most of the human cases had direct exposure to infected poultry in live bird markets with likely airborne virus exposure (Lai et al., 2016, Zhou et al., 2016). Experimental studies confirmed that processing asymptomatic HPAI virus infected chickens generated aerosols and large droplets containing the virus and exposure of ferrets (the model for human infection) to the same air space, transmitted, and caused fatal infections (Bertran et al., 2017). There is one case of human avian influenza virus infection associated with defeathering infected wild swans in Azerbaijan (Gilsdorf et al., 2006).

Changing Ecology and Epidemiology

Individual avian influenza (AI) virus strains vary in their ability to infect, transmit and cause disease and death in different bird species (Swayne et al., 2020). The LPAI viruses are maintained in wild aquatic birds, especially in dabbling ducks, and the virus must have prolonged direct or indirect contact for transmission and requires genetic adaptation to establish endemic infections in domestic poultry (Figure 2). The LPAI viruses replicate in respiratory and intestinal tracts causing low mortality (Figure 2). The HPAI viruses arise from mutation in the hemagglutinin of H5 or H7 LPAI viruses while circulating in terrestrial poultry (Figure 2). Once a LPAI or HPAI virus are adapted to terrestrial poultry, they have been rarely demonstrated to transmit back to wild aquatic birds (Figure 2).

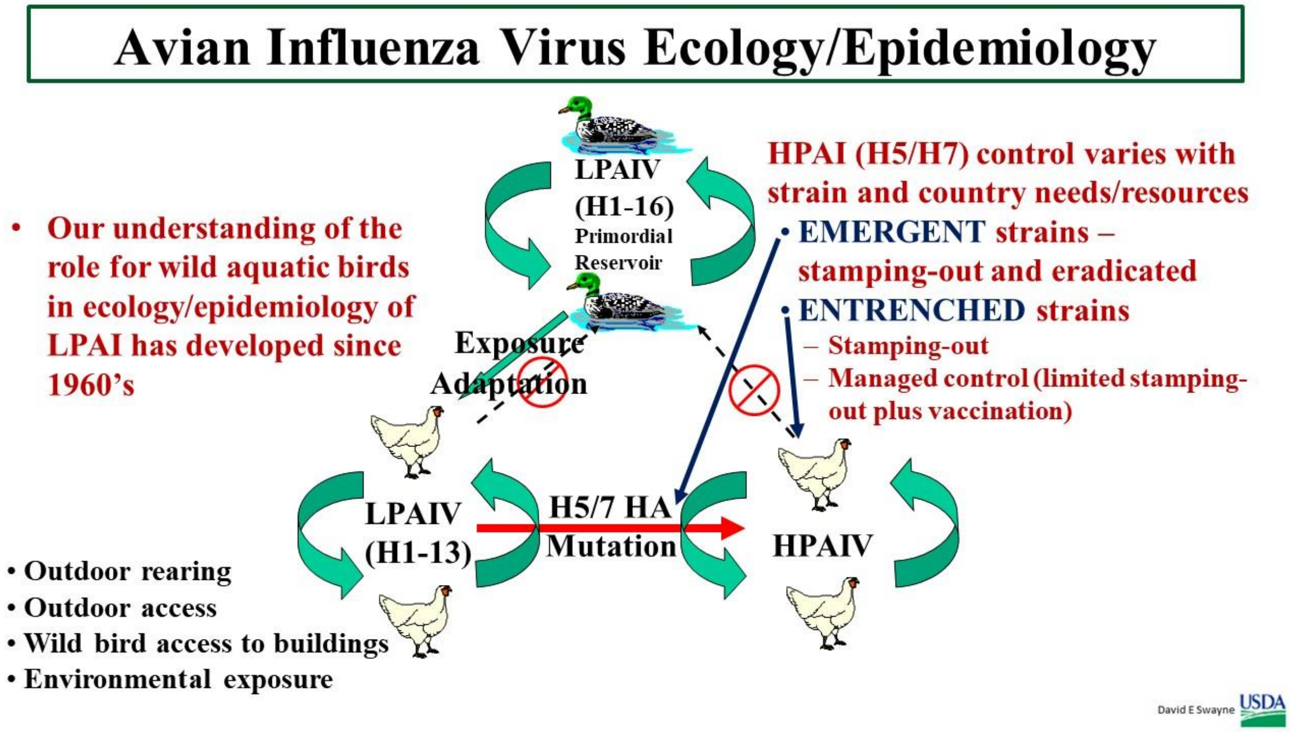


Figure 2. Ecological and epidemiological relationship between wild birds and poultry with avian influenza viruses developed between 1960s and late 1990's.

The HPAI viruses causing poultry outbreaks have varied epidemiological features. Of the 44 HPAI outbreaks, 41 followed the process of introduction of H5 or H7 LPAI virus from wild bird reservoir, adaptation to gallinaceous poultry and mutation to HPAI virus followed by eradication through stamping-out programs, mostly on the first or second flock, but some required extended periods of over a year for stamping-out policies to overcome the field spread and achieve eradication (Figure 1). In three HPAI outbreaks, the stamping-out program was not effective at eradication because of continued introduction of naïve poultry into the affected country or zone, and the inability to quickly identify and quarantine affected flocks for depopulation. These three virus lineages still circulate and cause HPAI outbreaks in poultry (Figure 1).

The LPAI viruses can cause disease in poultry. The most widespread and economically important is the H9N2 Eurasian lineage of LPAI virus. This virus has spread in commercial and live bird market system reared poultry in Asia, the Middle East, and North and West Africa costing billions in economic losses from respiratory disease and drops in egg production, and mild to moderate mortality losses (Swayne et al., 2020). These H9N2 LPAI viruses have diverged into multiple antigenic lineages with the need for multiple inactivated vaccines strains for protection, based on national or regional viruses. The largest of the 44 HPAI outbreaks has been caused by H5Nx Gs/GD lineage of Eurasian HPAI virus which began in 1996 with a report of fatal disease in domestic geese in China and has evolved through mutations into multiple genetic clades of the viral hemagglutinin gene (Xu et al., 1999, Sims and Brown, 2017). Historically, Gs/GD HPAI cases were initially H5N1, but assortment of the gene segments with other HPAI or LPAI viruses has produced H5N2, H5N3, H5N5, H5N6 and H5N8 HPAI viruses. In addition, many of the internal gene segments have reassorted.

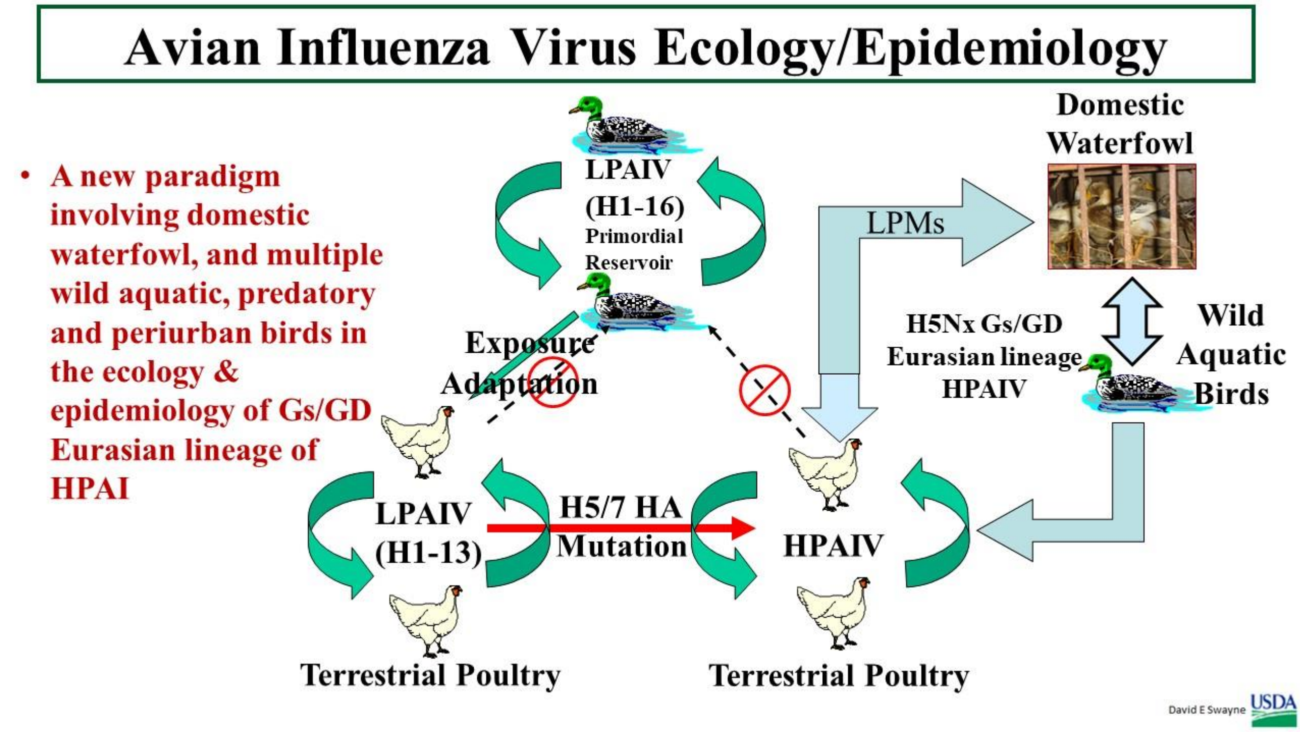
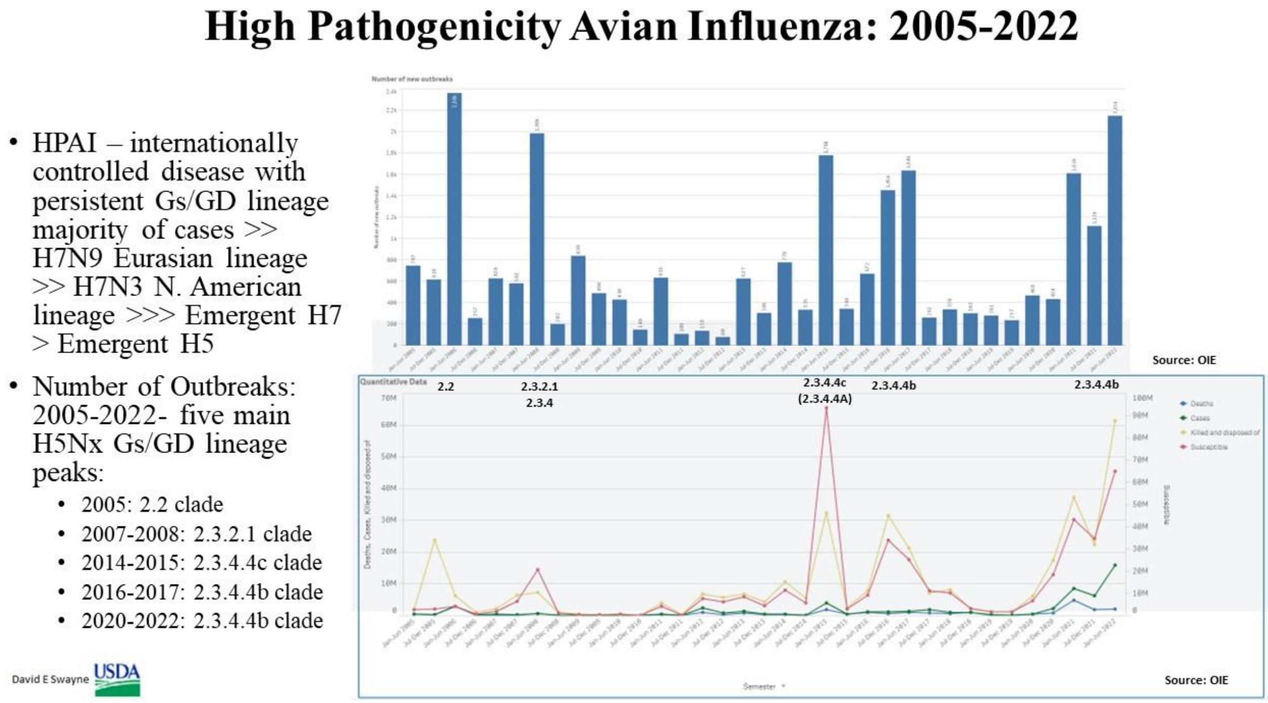


Figure 3. Change in ecological and epidemiological relationship between wild birds and poultry with H5Nx Gs/GD lineage of Eurasian HPAI virus after 1996.

Over the past two decades, Gs/GD lineage of HPAI virus infections has changed phenotypically in wild birds from resistance to infection to some viruses causing severe disease and mortality, and with a paradigm shift as wild birds becoming involved in both short and long distance spread of the virus (Figure 3). Intercontinental spread and large outbreaks of Gs/GD lineage HPAI viruses have occurred in poultry in North America, Africa, Middle Eastern and European Union countries. There have been five main waves of intercontinental spread: 1) clade 2.2 (2005), 2) clade 2.3.2.1 (2008), 3) clade 2.3.4.4c (previously termed 2.3.4.4A) (2014-15), and 4) clade 2.3.4.4b (2016-2017 and 2020-2022) (Figure 4) (Sims and

Brown, 2017). Since 2012, the 2.3.4.4 clade viruses have diverged into eight genetic subgroups: a-h with major outbreaks of 2.3.4.4b occurring across Asia, Europe, and Middle East in late 2020 and early 2021.



High Pathogenicity Avian Influenza: 2005-2022

- HPAI – internationally controlled disease with persistent Gs/GD lineage majority of cases >> H7N9 Eurasian lineage >> H7N3 N. American lineage >>> Emergent H7 > Emergent H5
- Number of Outbreaks: 2005-2022- five main H5Nx Gs/GD lineage peaks:
  - 2005: 2.2 clade
  - 2007-2008: 2.3.2.1 clade
  - 2014-2015: 2.3.4.4c clade
  - 2016-2017: 2.3.4.4b clade
  - 2020-2022: 2.3.4.4b clade

David E Swayne

Source: OIE

Source: OIE

Figure 4. Summary of high pathogenicity avian influenza, 2005-2022 by 6-month periods. Source OIE.

Since 2020, most of the 2.3.4.4b viruses have been H5N1, H5N8 and H5N6 with H5N1 spread intercontinentally between Asia, Europe, Africa, and North America. The current outbreaks include migratory aquatic birds, raptors, backyard and village poultry and commercial poultry, and a few human infections. Over 5771 outbreaks affecting 157 million poultry have been reported to World Organization for Animal Health since 2020.

Mitigation

Poultry, wild birds and humans are intertwined when developing methods to prevent and control avian influenza, especially HPAI. For example, the H5Nx Gs/GD lineage HPAI viruses spread down the Pacific Flyway by wild birds in North America, and in early spring of 2015, reached the Midwest USA (Lee et al., 2018). Molecular analysis, supported by epidemiologic findings, suggested that affected farms in the Pacific Flyway, and early cases in the Midwest, were largely due to point source introductions from wild birds, while later cases in the Midwest were the result of secondary spread between farms (Lee et al., 2018). Introduction from backyard to farms was not identified. These events highlight the benefits of a rapid stamping-out response and emphasize the need for improved biosecurity to prevent introduction of HPAI viruses from poultry or wild birds.

Poultry Vaccination. Control programs in some low and transition income countries include use of vaccines along with stamping-out of infected flocks to maintain food security, while high income countries have used stamping-out programs without vaccination. The use of vaccines, along with changes in marketing practices was associated with reduction in human cases in Vietnam (Sims and Brown, 2017).

Historically, HPAI in poultry has been met with stamping-out programs leading to eradication of most outbreak viruses. However, stamping-out strategies alone are unsustainable against entrenched or re-introduced HPAI viruses (e.g., H5Nx Gs/GD strain) and control efforts could benefit from broader use of vaccines. In a few poultry outbreaks, the HPAI viruses have become entrenched, with a few countries utilizing vaccines as an additional management tool which has led to the control of the virus in some countries and elimination in others. Globally, vaccination has not been entirely accepted as a control tool, but scientific data produced over the past decade has supported a greater use of vaccines in control and eradication programs. Influenza virus mutation, i.e., drift in the hemagglutinin, has generated antigenically divergent field viruses which need multi-national, national & regional seed strains for protection. Fortunately, newer vaccine platforms are more easily updated to achieve a closer match and improved protection. This has been augmented by the adoption of accelerated national regulatory processes for approval and deployment of revised or updated seed strains in non-replicating vaccines such as RNA-particle, DNA and inactivated whole influenza A virus vaccine platforms. Also, some technologies favor mass field vaccination that could eliminate the need for capture and vaccination of individual meat chickens, and reducing labor cost, simplifying logistics and reducing the cost of vaccination. These include vectors based on Newcastle disease virus and other avian paramyxovirus, and Marek’s disease herpesvirus (rHVT). The rHVT is especially favored as it can be automated in the hatchery for *in ovo* or post-hatch vaccination and persist to replicate and produce long term immunity. Finally, deletant mutants of influenza A virus have a restricted growth, reduced reassortment potential and reduced transmissibility hold promise for immunization in the field by spray vaccine application.

Changes in Poultry Slaughter. In developing simple mitigation strategies, use of vaccinated chickens in simulated home slaughter studies reduced the amount of virus replicating in birds and thus prevented generation of airborne virus during slaughter (Bertran et al., 2018). If the first processing step (i.e., kill and bleeding step) was conducted in a plastic bag, halla cooking pot with lid or plastic bucket with modified lid, a great reduction to complete elimination of airborne virus was seen with a reduction or prevention of transmission occurred. Furthermore, if the defeathering step was conducted by manual defeatherer, the quantity of airborne particles and airborne virus was less than compared to mechanical defeathering. A simple pictorial education program was developed and modified from focus group input to communicate the modified home slaughter program in Egypt. This program was communicated through the Food and Agriculture Organization to local organizations to reduce risk for human HPAI virus infections as a One Health outcome.

Summary

The LPAI viruses are maintained in migratory aquatic birds, especially dabbling ducks, and have been transmitted to poultry through direct or indirect exposure and once adapted, they have circulated and have been maintained in some poultry production systems. Some of the H5 and H7 LPAI viruses have mutated to HPAI viruses. Both have produced negative impact on poultry health and welfare. Avian influenza viruses have caused limited direct infections in humans and the Gs/GD Eurasian lineage has infected and caused disease in some wild bird species. Control of avian influenza viruses requires coordinate, collaborative work between veterinary, environmental and public health specialist.

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Assessing and Improving Poultry Welfare Beyond Compliance, a Multistakeholder Initiative (K. Schwean-Lardner & A.M. Neeteson)

Karen SCHWEAN-LARDNER and Anne-Marie NEETESON

Karen SCHWEAN-LARDNER, University of Saskatchewan (CA), Anne-Marie NEETESON, Aviagen (NL)
Karen.Schwean@Usask.Ca and aneeteson@aviagen.com

“Poultry welfare” as a term has evolved over time to go beyond compliance with regulation and required minimums for basic care to now encompass every step taken to create a prosperous environment for the birds and the people who care for them. Immense scientific research has taken place in recent decades that has unlocked management advancements based on improved understanding of poultry genetics, nutrition, housing, and more. This work also revealed the importance of assessing poultry welfare through outcomes-based measures and observations to capture the management practices that truly support greater positive welfare outcomes in poultry.

However, limited resources are available to put this scientific understanding into practice. While there are innumerable welfare programs/standards available, there is a need for a comprehensive list of outcomes-based key welfare indicators (KWIs) with standardized measurement instructions that could be used in different poultry species and at different production stages. Now, a new resource created by the International Poultry Welfare Alliance, the IPWA KWI Guide, is being released to the public to support improvements in poultry welfare and bring an opportunity for consistency in the way poultry welfare is assessed globally. This guide includes KWI assessment options, reasoning, and observations — all broken out by the specific needs of commercial broiler chickens, layer chickens, and turkeys.

IPWA was assembled as a multistakeholder initiative to create resources and drive improvements in poultry welfare based in proven science and shared values across entire global supply chains. IPWA, whose membership served as the primary authors, is made up of over 500 production managers, welfare program supervisors, researchers, business owners, poultry buyers for restaurants and retailers, veterinarians, and other professionals actively involved in the creation, implementation, and/or verification of welfare protocols for poultry. Their expertise and current work covered every poultry market in the world and shaped what is included in this reference. The IPWA KWI Guide also underwent a rigorous academic review and revision process, bringing together research experts from the world’s leading institutions and growing poultry research programs, including several experts also involved in the World Poultry Science Association. The latest update on this project and access instructions will be provided to the World Poultry Congress.

PS 3

The bird and its microbiota

The intestinal microbiota, a critical actor in the development of the immune response (B. Kaspers)

Bernd KASPERS

B. Kaspers (1), S. Härtle (1), S. Lettmann (1), B. Schusser (2), C. Schouler (3), P. Velge (3)

1) Department for Veterinary Sciences, LMU Munich, Germany
2) Reproductive Biotechnology, School of Life Science Weihenstephan, TU Munich, Germany
3) INRA, Infectiology and Public Health, Nouzilly, France
Corresponding author: kaspers@lmu.de

Abbreviated Title: Chicken microbiome and immune system

Summary

Development of the immune system is controlled by genetic programs and environmental cues. While mammals acquire the initial gut flora from their mother this is not the case in modern poultry production. Whether this impacts on the development and functional maturation of the immune system is largely unclear. We therefore compare immune system development in sterile (germ free, GF), mono- and tetra-reconstituted and SPF birds as well as birds raised in the presence of a maternal flora. No significant differences were evident in the development of selected parameters of the innate immune system between the groups. In contrast, by using microarray-based gene expression studies striking differences were observed in the adaptive immune system. In particular, mRNA abundance of genes associated with the B-cell system was significantly reduced in GF birds. This was reflected by highly reduced numbers of B-lymphocytes in the gut, which was paralleled by a complete absence of IgA production. The B-cell compartment and IgA production were slightly restored by mono-reconstitution and further enhanced in tetra-reconstituted and SPF birds. However, development of the B-cell system was greatly retarded in all groups in comparison with birds that acquired a maternal flora on the day of hatch. In contrast, neither B-cell maturation in the bursa of Fabricius nor circulating B-cell numbers were affected. These data predict that molecular cues induced by microbial colonization attract circulating B-cells into the mucosal tissue and regulate maturation towards IgA producing cells. Interestingly, these signals do not only activate homing and maturation of the B-cell compartment but also maturation of the epithelial IgA transporter system as poly-Ig receptor (PIGR) expression was very low in germ free birds but induced in response to microbial colonization. Identification of these microbiota derive signals will be an important future task.

Key words: chicken, microbiome, immune system, maturation, gut

Introduction

The mucosal surface of the gut is a fascinating structure. A single epithelial barrier separates the intestinal content from the body. The intestinal content does not only consist of digested feed but harbors a highly complex microflora called the microbiome. Consisting of bacteria, fungi and archaea and some poorly defined viruses the microbiome has been estimated to be made up of more than 1000 different species collectively representing more than 100-fold more cells than the vertebrate host itself does. The microbiota is well known for its capability to digest plant products which otherwise would be inaccessible for the vertebrate as a nutrient source. However, research in recent years has demonstrated that the microbiome affects not only metabolic processes but also the development and function of the immune system (Belkaid and Hand 2014). Conversely, the composition of the microbiome seems to be modulated by the immune system. This cross-talk between the bacterial flora, the intestinal epithelium and the immune system has become a research focus in immunology over the last 10 years. Immunologists have used germ free mouse models, gnotobiotic mice and mice treated with antibiotics to get completely sterile animals or mice with a defined microbiome. This work has led to a wealth of information in mouse models (Kamada et al. 2013) while knowledge in humans and domestic animal species is still limited.

The avian immune system

The avian immune system is largely similar to its mammalian counterpart, even though some striking differences are found such as the absence of lymph nodes and the presence of a unique organ for B-lymphocyte development only existing in birds named the bursa of Fabricius (Nagy et al. 2022). Two major arms of the immune system have been defined which are called the innate and the adaptive immune system. The innate immune system provides a rapid response to pathogenic challenge while the adaptive immune system is responsible for sustained pathogen control and immunological memory. Both systems are made up of cellular and soluble components some of which have been known for a long time including macrophages and heterophils, complement components, and antibodies. Others have been characterized more recently in mice but are still poorly defined in birds (e.g. T-lymphocyte subsets or innate lymphoid cells). The two arms of the immune system closely interact with each other to provide adequate protection and communicate with each other through cell surface molecules and soluble factors collectively called cytokines. Cytokines play a critical role in the regulation of immune response to pathogens, vaccination, in immuno-regulation, and in the control of inflammation. Since the initial publication of the chicken genome significant progress has been made in avian immunology. Genomic data provides sequence information for genes involved in immuno-regulation and the function of the well-defined lymphoid tissues of the spleen, thymus, and the bursa of Fabricius. In contrast, mucosal tissues of the lung, reproductive tract, and the gut are less well understood.

Development of the gut associated innate immune system of chickens

Using immunohistology, holistic gene expression analysis, and functional assays we have investigated the development of the gut immune system from hatch to seven weeks of age in both broilers and layers. Macrophages are readily found in large numbers from the day of hatch throughout the gut. Their number increases in parallel with gut growth. Macrophages are located in the lamina propria and are thus well positioned to rapidly detect invading pathogens or epithelial damage. Studies in *Salmonella enterica* infection models of day-old chicks have revealed that these macrophages are fully functional and respond to pathogen invasion with the secretion of inflammatory cytokines and chemokines to attract more phagocytes into the mucosal tissue which help to clear the infection. Within hours of infection the chemokines interleukin-8 (IL-8) and CCL-16 are produced. They attract heterophils and macrophages from the blood, respectively. In addition, effector cytokines regulating the anti-microbial response such as interferon-γ have been detected by RT-PCR. Thus, macrophages and other components of the innate immune system are present and functional at hatch when the bird first encounters microbial organisms in its environment. In addition to the cellular components of the innate immune system, soluble components are found early after hatch. These include several acute-phase molecules of the pentraxin family and anti-microbial peptides of the defensin family (Cuperus and others 2013), which have been demonstrated by RT-PCR analysis. However, their functional role in the avian intestine has not been studied due to the lack of appropriate animal models deficient on one or several of these factors. From work in mice, it has become clear that anti-microbial peptides are produced by epithelial cells of the crypts called paneth-cells and are secreted into the mucus layer. Bacteria invading the mucus barrier get in contact with these factors and are efficiently lysed. Most authors agree that paneth-cells (Nile and others 2004) are absent in chickens and even though defensin specific mRNA is found in gut tissue samples it is largely unclear where they are produced and which mechanisms elicit their synthesis and secretion.

While the innate immune system seems to be functional at hatch the components of the adaptive immune system are absent at that time point. Developmental studies have shown that B-lymphocytes, the producers of antibodies, start to emigrate from the bursa of Fabricius around hatch. Similarly, emigration of T-lymphocytes from the thymus starts shortly before hatch but major waves of cell migration are only observed days after hatch (Ratcliffe 2006). Importantly, both populations of lymphocytes are rare in the gut tissue during the first two weeks after hatch. Subsequently, more and more lymphocytes colonize the mucosal tissues and formation of lymphoid aggregates is found. Lymphocytes can be demonstrated in the lamina propria and in the epithelium as intro-epithelial lymphocytes. The latter population is made up of T-cells specialized in the control of virally infected cells. They are called cytotoxic T-lymphocytes (CTL) and are characterized by the expression of the cell surface protein CD8. T-helper cells (expressing the CD4 molecule) are more restricted to the Lamina propria and are found in close proximity to lymphoid aggregates. B-lymphocytes start to colonize the gut at nearly the same time. They form organized lymphoid structures called Payer's patches and caecal tonsils.

Studies performed more than 50 years ago with birds raised under germ free conditions have shown that bacterial colonization has a significant influence on the maturation of the gut associated immune system (Thorbecke et al., 1957). This observation has been confirmed recently in germ free mouse models and in our own studies with germ free chickens. To investigate the role of the microbiota, we hatched groups of birds which were maintained sterile or received one (*E. coli* Nissle) or a mixture of four strains of bacteria (*E. coli, Lactobacillus, Enterococcus, Clostridium*). These birds were compared with chickens housed under SPF conditions. Even in the complete absence of microbiota the innate immune system (as far as investigated) developed normally. No difference was observed with regards to colonization by macrophages and micro-array studies showed undisturbed expression patterns for several genes of the innate immune system. In striking contrast, the development of the adaptive immune system was strongly impaired. B-cells were absent in the lamina propria of four weeks old birds despite normal B-cell development in the bursa of Fabricius. B-cell regions of the caecal tonsils were hugely underdeveloped and did not contain germinal centers, which are essential for the production of IgA and IgY antibodies. Consequently, IgA production was absent in germ free birds in the gut and in the serum. A similar picture was observed in the T-cell system with the absence of both CD4+ and CD8+ T-cells in germ free animals. Colonization with *E. coli* alone did not revert the histological picture but lead to the production of IgA in serum. These antibodies were, to a large extend, *E. coli* specific. Birds colonized with four bacterial strains showed significant maturation of the adaptive immune system in the gut. This was most pronounced in the caecal tonsils were germinal centers formation and IgA production were observed.

The described observations were further supported by our gene expression analysis. Comparison of micro-array data from germ free and SPF birds showed higher expression of a large number of genes associated with immune function and developmental processes in SPF chickens. Most strikingly, genes known to be critical in B-cell maturation were found to be far less abundant in germ free birds reflecting the phenotype observed by immune-histology. AID, an enzyme essential for immunoglobulin class switching from IgM to IgA and IgY was weakly expressed in germ free birds but significantly higher expressed in mono-reconstituted chickens. A further increase was detectable in tetra-reconstituted animals, which showed expression levels equivalent to SPF birds.

Under physiological conditions, chickens get immediately in contact with the hen's microbiota. To compare the above-mentioned conditions with a flora acquired under natural conditions, additional experiments were performed. White leghorn chickens with the same genetic background were either placed under SPF conditions or in an aviary which was used by laying hens for the last 72 hours (conventional birds). Comparative microbiome analysis by 16S rRNA gene amplicon sequencing revealed a much higher microbiome diversity in the letter group, which was reflected by enhanced gut immune system development. Most strikingly, IgA antibody levels in plasma increased three-fold over those in the SPF group. Interestingly, vaccination did not increase total IgA plasma levels in both group beyond the levels of unvaccinated birds.

Micro-array data also indicated that mRNA abundance of the polyIg-receptor was significantly lower in GF birds than in SPF and conventional birds. This receptor is expressed on the basolateral side of gut epithelial cells and is essential for the transport of IgA from the Lamina propria into the gut lumen. Here IgA binds to pathogenic bacteria thus preventing their invasion. In addition, mucosal IgA was shown in mouse models to modulate the gut flora in favor of the host, underlining its importance for maintenance of gut homeostasis. Since GF birds expressed very low mRNA levels of the polyIg-receptor and lack both a microbiota and gut B-cells at the same time, we asked the question if receptor expression is regulated by signals derived from the B-cells or from gut microorganisms. To address this question, we made use of a unique chicken model, the B-cell knockout birds recently published. Since these birds completely lack B-cells but have a normal microbiota, polyIg-receptor expression should be downregulated if B-cell derived signals were the critical regulators. Our experiments showed that this was not the case and that microbial colonization and gut-derived signals are essential for receptor expression. We cannot discriminate whether microbial metabolites or microbial fermentation products are responsible for this effect. Nevertheless, these studies clearly show that the microbiota also affects the function of the gut epithelium in chickens and regulates the B-cell system in a complex way by acting simultaneously on different cellular compartments in the gut.

In order to identify gut derived signaling molecules, future work has to identify, isolate and culture as many members of the chicken microbiota and elucidate their metabolic pathways. First steps towards this end have been made recently and functional studies are under way (Zenner et al. 2021). One such molecule is well known in poultry nutrition, the short chain fatty acid (SCFA) butyrate. Besides its well-established importance as an energy source and as a regulator of microbiome composition, recent work in mice identified cell membrane expressed receptors for butyrate and other SCFA (Tan et al. 2017). Binding of butyrate induces an anti-inflammatory pathway in macrophages and thus promotes an anti-inflammatory condition in the gut. In addition, it enhances the secretion of antimicrobial peptides by gut cells again supporting the regulation of microbiota composition. Both effects of butyrate on macrophages were also shown for chicken cells. Furthermore, intestinal derived butyrate increases the number of so-called regulatory T-cells (Treg). These cells are important regulators of anti-inflammatory responses in the gut through the secretion of IL-10 and TGFβ. While the existence of this T-cell population was shown earlier, their precise identification and functional characterization as well as their response to microbiota derived signaling molecules is still unknown. Treg-cells express a unique transcription factor called FoxP3, which is characteristic for this cell type in mammals. FoxP3 was believed to be absent in the chicken genome but our recent work identified chicken FoxP3 (Burkhardt et al., 2022) and thus opens new ways to investigate the complex regulatory network in the gut.

Conclusion

The development of the gut immune system is controlled by both genetic and environmental factors. The innate immune system develops early in ontogeny and is present at hatch and thereby able to protect birds during the critical phase of sudden encounter with microbes after hatch. Its subsequent development is not strongly influenced by the microbiota but it becomes strongly activated in response to pathogen invasion. In contrast, development of the adaptive immune system is significantly influenced by bacterial colonization of the gut. Absence of a microbiome leads to severe developmental defects with a lack of B- and T-lymphocytes in the gut mucosa and the absence of antibody formation.

Our studies strongly indicate that full maturation of the gut immune system requires a complex flora. However, the precise composition of an optimal flora is still unknown. Under natural conditions hatchlings get in immediate contact with the maternal flora while birds hatched under commercial poultry production conditions are hatched essentially sterile. It is still unclear how these conditions influence the development of the mucosal immune system, the capability to control pathogenic microorganisms, and animal health and welfare. Interestingly, mouse studies have shown that nutrients and fermentation products also influence the functional status of the mucosal immune system either directly or through mediators secreted by the epithelial cells. The role of the epithelium, the mucus barrier, and macro- and micro-nutrients on the avian immune system has not been investigated to date but should be considered in future studies.

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Highlights from studies on poultry microbiome: what is missing to turn research into innovation? (A. De Cesare)

Alessandra DE CESARE

Department of Veterinary Medical Sciences, *Alma Mater Studiorum*, University of Bologna

Via Tolara di Sopra 50, 40064 Ozzano dell'Emilia (BO), Italy
alessandra.decesare@unibo.it

Abbreviated title: Turning poultry research into innovation

Summary - Advances in genomic sequencing, multi-omics and computational approaches are empowering scientists with tools to decode the microbiomes circulating in the poultry food chain and their metabolic potential. To fully exploit such potential in innovative and sustainable solutions able to enhance the economic, environmental and social sustainability of circular broiler productions, as well as safety of poultry meat, the positive microbiome networks circulating in the poultry food chains must be fully mapped under a representative number of categorized environmental and production conditions. Modelling the associations between intrinsic and extrinsic drivers of animal health and poultry meat safety, during farming, transport and slaughtering allows to identify the microbiome settings associated to healthy animals and sustainable productions. However, the achievement of cutting-edge results and their translation into valuable innovations requires a systematic metadata collection feeding artificial intelligence and machine learning algorithms and the validation of algorithms predictions in commercial conditions. All these needs can be satisfied making possible a strong cooperation and trust between researchers and poultry producers, building together the economic, environmental and social sustainability of the future poultry productions.

Keywords: Broilers, metagenomics, multi-omics, functional genes, antibiotic resistance, feed additives, big data.

Background - Research projects facing different aspects of poultry production have been funded for decades. However, the translation of the results into innovations and strategies to solve productivity and poultry meat safety related issues is always challenging.

Aim - The aim of this paper is to look at recent research studies and projects dealing with broiler production and identify what is still needed to promote the turning of research results into innovations able to solve urgent problems for the poultry sector in a food production world moving from horizontal chains to circular economies.

How to identify the positive microbiome networks circulating in broiler productions - The broiler production includes a number of steps. Among them the rearing of the animals in the poultry houses and the processing of the carcasses in the slaughterhouses are the most crucial in terms of impact on the economic and environmental sustainability of poultry productions as well as safety of poultry meat. In the poultry house the birds should stay healthy and gain good productivity scores, while avoiding their treatment with antimicrobials. To these aims, beside a continuous improving of biosafety measures, probiotics (De Cesare et al., 2020), prebiotics, organic acids and enzymes (Brugaletta et al 2022) are added to the feed or to the litter (De Cesare et al., 2019). The microbiomes colonizing the chicken gut are certainly affected by the diet and the administration of feed additives, but they are also influenced by the other microbiomes circulating in the poultry house and in the surrounding environment. However, the impact of the latter has not been investigated. In the same way, the microbiomes associated to broiler carcasses and poultry meat are affected by the flock of origin but also by the microbiomes circulating around the animals, during transport and at the slaughterhouse. The try to clarify how the microbiomes interact one with the other in the poultry food chain in order to enhance positive microbiomes networks, the consortium of the H2020 funded project named CIRCLES is mapping the microbiomes circulating in selected broiler farms and slaughterhouses. In each of the farms, the microbiomes investigated are those associated to caeca droppings, litter, drinking water, feed, air, soil outside the poultry house and farmers. In the slaughterhouses the microbiomes addressed are those of the carcasses and corresponding gut contents. The composition of all these microbiomes is modelled in association with a number of registered and measured environmental, performance and microbiological parameters to identify those microbiomes consistently found in positive scenarios, meaning in farms with healthy and well performing animals, where treatments with antibiotics are not needed and resulting in meat of good quality and microbiological standards. The outputs of such a coordinated research strategy will finally result in the identification of microbiome-based interventions to enhance animal health and poultry meat safety.

The importance of the selection of the genomic method to be applied in microbiome studies - In the CIRCLES framework the first research question to answer has been what is the best approach to deeply investigate the microbiomes circulating in the poultry food chain. To answer this question Durazzi et al 2021 investigated the reliability of two sequencing strategies named metagenomics (i.e., whole shotgun metagenomic sequencing) and metataxonomics (i.e., 16S rRNA gene sequencing) for bacteria profiling, studying the chicken gut as a model system. The results showed that in comparison to metataxonomics, metagenomics offers a deeper mapping of the microbial populations in a sample. The experimental conditions included in the study concerned two compartments of gastrointestinal tracts (i.e., ileum and caeca) and two sampling times (14 and 25 days). The relative abundance distributions obtained with the two sequencing strategies were compared and tested for their capability to distinguish between the experimental conditions. The results showed that 16S rRNA gene sequencing detects only part of the gut microbiome community revealed by shotgun sequencing. Specifically, when a sufficient number of sequences is available, shotgun metagenomics has more power to identify less abundant taxa than 16S sequencing. However, those less abundant genera detected only by shotgun sequencing are biologically meaningful, being able to discriminate between the experimental conditions as much as the more abundant genera detected by both sequencing strategies.

The potential of the multi-omics approach in microbiome studies - Beside the taxonomic composition of a sample, the shotgun metagenomic output provides the quantification of the relative abundance of the functional genes in the sample. Although the presence of a functional gene at a high relative abundance is a good proxy to assume that it will be expressed, a key research question to answer is how to validate the shotgun metagenomic results. The best strategy is certainly plan research studies including a multi-omics approach, meaning coupling shotgun metagenomics with a second, or even a third omic technique, as metabolomic, or transcriptomic. Brugaletta et al 2022 elucidated the mechanisms of action of a dietary muramidase administered to broiler chickens analyzing performance traits, welfare indicators, breast muscle myopathies, caecal microbiome, and caecal and plasmatic metabolomes. The shotgun metagenomic results demonstrated a lower abundance of Firmicutes in the group fed with muramidase and a corresponding decrease in genes associated with peptidoglycan biosynthesis pathway compared to the control. The reduction in genes involved in amino sugar and nucleotide metabolism pathway, in which N-Acetylglucosamine is directly involved, has been considered as indicator for the inhibition of bacteria with high peptidoglycan synthesis capacity. In the treated group, the decrease in abundance of genes of starch and sucrose metabolism pathway measured by shotgun metagenomics has been linked to the reduction in fermentations-deriving organic acids, such as acetate, ferulate, and formate measured by metabolomics. Moreover, the enrichment in genes linked to glutathione metabolism pathway has been associated with the hypoxanthine drop at caecal level. Finally, the higher abundance of caecal Bacteroidaceae has been interpreted as the driver increasing the supply of propionate for the hepatic gluconeogenesis in the treated birds thereby leading to the observed rise in plasmatic energetic compounds, such as pyruvate, 2-oxoglutarate, and glucose.

The importance of longitudinal studies and validation trials in commercial conditions to translate microbiome research results into innovations - The identification of the biological link between microbial groups, identified as signature species, functional genes, quantified as characterizing features in different experimental groups, and the amount of specific metabolites in animal or food systems, require complex bioinformatic and biostatistics tools as well as strong computational expertise and infrastructures, because the inputs of different omics methodologies must be transformed in such a way to be comparable and modelled in association with relevant metadata. This means that the research groups involved in these studies must include a number of different expertise. Moreover, since even the most sophisticated analysis might suffer of numerous biases, it is crucial to organize longitudinal trials, including at least three sampling points along the rearing cycle. Finally, the outputs of the longitudinal modelling of the microbiomes in association with each set of metadata should be validated in trials performed in commercial conditions, requiring a strong cooperation between research teams and poultry companies.

The neglected role of the environment in the emergence and spread of AMR - The functional genes which can be mapped by shotgun metagenomics include the genes coding for antimicrobial resistance (AMR), all together representing what is named resistome. A recent EFSA opinion (EFSA 2021) assessed the role played by food-producing environments in the emergence and spread of AMR causing each year 33,000 human deaths in the European Union (Cassini et al., 2019). The use of antibiotics in animal food productions has been considered the primary cause of shedding of antibiotic resistant pathogenic and commensal bacteria, which can then be transferred to humans via several pathways, including the food-chains. Many initiatives and strategies have been put in place by both policy makers and producers to reduce the level of antimicrobials used in animal productions and as a possible consequence, in 2017, for the first time since 2011, the antimicrobial consumption in food producing animals was lower than in humans (i.e., 108.3 mg/kg vs. 130 mg/kg) (ECDC-EFSA-EMA, 2021). The 2021 EFSA opinion highlightsthat besides the prudent antimicrobial use, the most important measure to mitigate AMR in primary production and during slaughtering is to follow effective hygiene practices and biosecurity measures in order to reduce both the occurrence and transmission of pathogens and other microorganisms. The most relevant risks for public health identified in the poultry food chain have been *Salmonella* isolates, resistant to extended spectrum cephalosporins or fluoroquinolones, and *Campylobacter* strains, with high level fluoroquinolone resistance, which have been isolated in different environmental sources. Moreover, extended spectrum cephalosporins-resistant Enterobacteriales strains, and multidrug resistant *E. coli* and other Enterobacteriales with ESBL/AmpC plasmid-mediated genes and strains with high level fluoroquinolone resistance, have been detected in poultry environments (e.g., in rats, flies, wild animals, manure/litter). The results summarized in the EFSA scientific opinion highlight for the first time the need to perform harmonized environmental AMR monitoring and surveillance strategies and long-term longitudinal cohort studies on highest priority antimicrobial resistant genes and bacteria, involving also environmental exposure of food animals.

The correlation between microbiomes and resistome - In the European Union-funded EFFORT project, 178 poultry herds were sampled in 9 European countries, generating herd-level composite samples showing differences in the relative proportions of AMR within the same European country and between countries (Munk et al., 2018). The most represented drug classes among the poultry herds were tetracycline, macrolide, β -lactam and aminoglycoside. In the tested samples the resistome correlated significantly with the bacterial composition ($P = 0.001$) and this explains why is so important to try to reduce the occurrence and dissemination of microorganisms applying strong biosecurity measures. This study demonstrated that the country level veterinary use obtained from the ESVAC was positively associated with AMR in poultry. This association has been previously demonstrated also testing the AMR in isolates cultivated in standard culture media. However, the use of shotgun metagenomics for the surveillance of the resistome circulating in the poultry food chains in association with relevant metadata is a more holistic strategy able to drive the identification of the most promising strategies to decrease AMR in the future.

The importance of extending the microbiome based and AMR mitigation strategies up to the fork - De Cesare et al 2022 performed a pilot study to verify whether the efforts of raising chickens without the use of antibiotics made any difference in the microbiome of poultry meat consumers eat. To this end, the authors compared the microbiomes characterizing caeca and the corresponding carcasses of two groups of chickens reared on different farms, one conventional and one antibiotic-free. The results showed a clear separation between the taxonomic, functional and antibiotic resistant genes in the caeca of the birds reared in the conventional and antibiotic free farm. However, that separation was completely lost on carcasses belonging to the two groups. The antibiotic-free production resulted in statistically significant lower antimicrobial resistance load in the caeca in comparison to the conventional production. Moreover, the antimicrobial resistance load on carcasses was much higher than in the caeca, without any significant difference between carcasses coming from the two types of farms. Overall, the results of this study highlight the need to reduce sources of microbial contamination and antimicrobial resistance not only at farm but also during transport and at the post-harvest level.

How to use big data and machine learning outputs to identify tailored innovations for the poultry sector - The metadata to collect in broiler research studies may include local weather data, air quality data, environmental parameters within and outside poultry houses and slaughterhouse, production performance parameters during farming, process parameters during slaughtering, trade and economic data. The collection and even on-line registration of such a huge number of metadata can be supported by the use of sensors, drones and other digital technologies all connected to the same database where the metadata, automatically or manually extracted, are associated with the results of the omics technologies all feeding artificial intelligence and machine learning algorithms. These algorithms can be applied to determine optimal nutrient composition of animal feed, evaluate animal management impacts, forecasting production outcomes in new scenarios (e.g., climate change or reduced use of antimicrobials), provide an early warning for emerging human, animal and environmental risks. The large-scale application of breakthrough multi-analyte technologies, metadata collection, artificial intelligence and machine learning algorithms and the validation of algorithms predictions in commercial settings can certainly increase the exploitation of research results in innovations able to solve complex problems for the poultry sector.

Conclusion - All in all, the value of the results collected using multi omics approaches in the poultry food chain is greatly increasing. However, the achievement of cutting-edge results and their translation into valuable innovations requires a systematic metadata collection feeding artificial intelligence and machine learning algorithms and the validation of algorithms predictions in commercial conditions. All these needs can be satisfied making possible a strong cooperation and trust between researchers and poultry producers, building together the economic, environmental and social sustainability of the future poultry productions.

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Breeding and nutritional strategies for sustainable poultry production schemes

Opportunities offered by genomic selection to breed for diverse production systems (J. Dekkers)

Jack DEKKERS

Jack Dekkers (1), Anna Wolc (1,2)
(1) Department of Animal Science, Iowa State University, 806 Stange Road, 239E Kildee Hall, Ames, IA 50010, USA
(2) Hy-Line International, 2583 240th Street, Dallas Center, IA 50063, USA.
Corresponding author: jdekkers@iastate.edu

Abbreviated title: Genomics for diverse production systems

Summary

Poultry production systems differ considerably across the globe, as do the markets that they target, beyond their broad categorization into broiler, layer, and dual-purpose production. Differences in production systems and markets are expected to increase as housing systems further develop to address societal demands, animal welfare, and animal health, and as environmental conditions change as a result of climate change. These differences introduce genotype by environment (GxE) interactions in terms of individuals traits (in particular for egg production and livability for layers) and breeding goals (differences in the relative importance of traits), resulting in the genetic ranking of lines or individuals within lines to change depending on the environment, production system, or market. This imposes challenges on breeding programs on how to best meet the needs of poultry producers across the globe. Poultry breeding programs have a pyramidal structure, with selection within multiple pure lines that must be kept in high-health nucleus herds and with single-cage housing systems for layers to allow data recording on individual hens. If the genetic correlation between performance in the nucleus and the field is low, selection of the pure lines for increased performance in a target production system or environment requires collection of relevant phenotypes on relatives of selection candidates in that environment and their use for genetic evaluation of the pure line selection candidates, either through pedigree or genomic prediction. Genomic prediction is a much more effective method to utilize such phenotypes and leads to greater responses to selection and lower rates of inbreeding. The presence of GxE between markets can be addressed by development of specialized lines or line combinations that are optimized for the target environment or by breeding lines that perform well across environments by broadening the breeding goal to include all traits that are relevant across the environments and by selecting for robust animals that are more adaptable to different environments. Collecting phenotypes in the target environments and using genomic predictions can increase genetic gain in either scenario. Genomic selection offers opportunities to utilize data collected on other lines and in other environments for genetic improvement of a given line. The optimal breeding scheme is conditional on trait genetic correlations between the environments, population size (risk of inbreeding in case of small specialized lines), and the cost of both phenotyping and genotyping, and must be evaluated on a case by case basis.

Keywords: GxE, genomics, breeding programs

Introduction

Poultry production systems differ considerably across the globe, as do the markets that they target, beyond their broad categorization into broiler, layer, and dual-purpose production. Differences in production and housing systems and markets are expected to increase as a result of climate change and as differing degrees of emphasis are placed on new perspectives, such as animal welfare, animal health, and sustainability. Because of these developments, hens are now expected to perform in a much wider variety of production systems. Birds of the same genetic background can be placed in floor systems, aviaries, free-range, or organic systems, each of them with different benefits but also specific challenges for the birds. In this wide variety of environmental conditions, hens are still expected to perform on a high level, with good efficiency, and supply consumers with high quality eggs. These differences can result in genotype by environment interactions (GxE), meaning that the genetic ranking of lines, breeds, and of animals within a line or breed is not consistent across environments or markets. This can include their ranking for individual traits based on estimated breeding values (EBV), as well as for overall performance, quantified by a total merit index (TMI) as the average EBV across traits weighted by the economic value of each trait (Dekkers and Gibson, 1998). Here, different environments (E) should be interpreted in a broad sense, including different management and production systems, diets, climatic conditions, and disease pressure, as well as differences in traits of interest and in economic values for traits when considering GxE for overall performance. At the level of individual traits, differences in trait definitions or phenotype recording can also result in GxE. When environments can be classified, presence of GxE can be quantified by the genetic correlations for the same trait recorded in two different environments (Falconer, 1993). If differences in environment are quantitative (e.g. disease pressure or climatic conditions), reaction norm models can be used to quantify GxE (de Jong and Bijma, 2002).

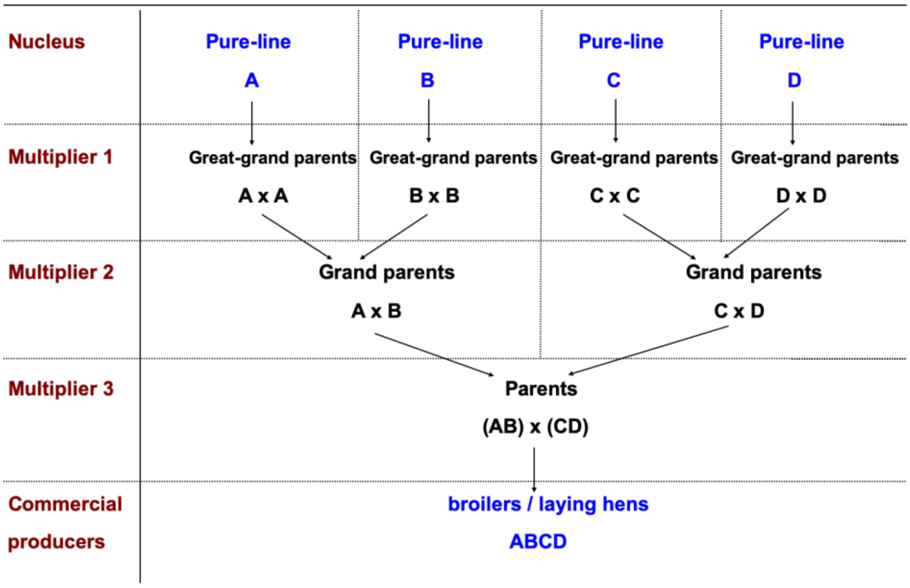


Figure 1. Typical structure of poultry breeding programs (Multiplier 1 is omitted in some cases to shorten the time to deliver genetic progress).

Presence of GxE imposes challenges on breeding programs on how to best meet the needs of their customers, as commercial breeding programs typically target an international market, with solutions ranging from developing or selecting lines or crosses that target a range of production systems, environments, or markets, to developing specialized lines or crosses. A comprehensive review of the history and recent developments in poultry production and in breeding programs is in Leenstra et al. (2016). The typical pyramidal structure of poultry breeding programs is illustrated in Figure 1, with most selection occurring in purebred lines in high-health nucleus herd.

Breeding program decisions on developing specialized lines are often economic in nature and depend, among others, on differences in expected responses to selection for breeding goals and for individual breeding goal traits when each production system has its own breeding program versus a single breeding program that targets a range of production systems, environments, or markets. Differences in responses to selection between these options depend on the genetic correlation between the breeding goals of the different production systems, environments, and markets, and on the correlation between the total merit selection indexes for the different breeding goals and are often restricted by the genetic correlations that exist among traits, which limits the ability to differentiate breeding programs to target specific markets. Development of breeding programs that target a range of production systems or environments also depends on the ability to select for animals that are more robust to environmental differences.

Selection decisions in breeding programs are driven by recorded phenotypes, which, through pedigree or genomics, are used to generate EBV for selection candidates for traits of interest. Thus, choices about which traits should be phenotyped on which birds and under which conditions are also important in the design and operation of breeding programs.

The purposes of this paper are to summarize the types and evidence of GxE in poultry production and discuss considerations for the choice between single or specialized lines to account for GxE and to meet industry and consumer needs. Many of these considerations are potentially impacted by genomics, genomic prediction in particular, as will be discussed. Our focus here will be on layer chickens but similar considerations apply to broilers and dual-purpose chickens, as well as other poultry and livestock species.

Evidence and types of GxE in layer chickens

Genotype by environment interactions in layers can arise on multiple levels such as type of housing, diet, climate, disease pressure, etc. In the past, cages provided fairly uniform conditions for birds across the globe, especially in larger facilities with automated control of light, humidity and temperature. However, there was still a difference between housing in single bird cages in nucleus breeding farms in order to provide optimal conditions for expressing egg production and quality traits and for individual phenotype recording, versus commercial conditions with multi-bird cages and typically much lower standards of biosecurity, less effective climate control, and lower feed quality. Competition is an important factor in multi-bird cages that creates substantial GxE compared to performance in single-bird cages. Muir (1996) showed that group selection can be used to improve welfare, livability, and production in multi-bird cages. They also showed that, when placed in single-bird cages, their group selected line was inferior to their commercial counterparts that were selected based on performance in single-bird cages, but the situation reversed if birds were placed in 12 bird cages, with an advantage for the group selected birds in terms of production but most notably in livability (Muir et al., 2014). This clearly illustrates GxE interactions and advantages of birds bred for specific production system.

On the strain level, Sighn et al. (2009) compared four strains of laying hens housed in cages and in a floor system and identified significant GxE interactions for egg production and body weight. In the German random sample test, Damme et al. (2012) found significant GxE only for egg production and also concluded that “differences between management systems were more important than differences between commercial strains, except for highly heritable traits such as egg weight.”. Icken et al. (2012) not only showed significant reranking of layer strains between cage and floor systems but also confirmed strong GxE within strains for peak egg production, with estimates of genetic correlations between systems ranging from 0.18 to 0.44. However, these correlations were much higher (0.56-0.97) for early production, which is strongly affected by the more stable trait of age at first egg. They also estimated a high genetic correlation for egg weight (0.78-1).

With climate change, the effects of heat on the ranking of layer breeds and strains has gained interest. Mathur and Horst (1994) used various methods to estimate the degree of GxE due to temperature and concluded that not all traits are impacted to the same degree by heat, with traits related to body morphology, body weight, and egg weight showing minimal levels of GxE, while egg production and egg mass were most significantly affected, with the magnitude of GxE effects exceeding across-environment genetic effects and genetic correlations less than 0.5 between production in temperate versus hot environments. Most of these results support the conclusion that laying rate and livability are most affected by GxE in layers (Hartman 1990).

At the level of overall genetic merit, GxE can also be the result in differences in the traits that are important in different production systems and markets and in differences in the economic values of traits. In developed countries, the focus is on egg number, egg quality, feed efficiency, and, more recently, adaptability to alternative production systems (robustness). In contrast, in developing countries, traits influencing livability are of major concern, including heat stress resistance, disease resistance, and ability to scavenge for feed.

Traits specific to alternative housing systems

A group of traits that are important for cage free production systems are traits related to perch and nest utilization. Perch utilization allows birds to take advantage of vertical space in the barn and also find a safe and comfortable place for resting. Greater nest utilization reduces the number of floor eggs, which create an issue not only because they require additional labor for manual collection, they also are more likely to be dirty and carry pathogen contamination and can, therefore, create food safety risks.

Habinski et al. (2017) identified differences between Columbian Rock, Rhode Island Red, and White Leghorns in perch utilization with age, indicating genetic differences in behavioral patterns between breeds. In Wolc et al. (2021), two lines of Rhode Island Red hens were recorded for perch utilization and percent of floor eggs. Percent of hens observed perching increased over the duration of the tests and the proportion of eggs laid on the floor decreased, suggesting that hens were able to learn to utilize perches and nests. Estimates of heritability for percent of floor eggs were very encouraging, at over 40% in both lines. Heritability of perching was about half of that but significantly greater than zero. Estimates of genetic correlations show that the hens that are genetically predisposed to using perches tend to lay fewer floor eggs. In principle, genomic methods could be used to more directly identify individuals that lay floor eggs in big group settings but at present the cost of such data collection is prohibitive.

Another new trait is winter garden utilization, which is also relevant to free range systems. With modern technology that allows tracking of the movement of individual hens, we can observe how much time the birds spend outside and how frequently they move between inside and outside (Icken et al., 2008, 2009). New technologies such as rfid tags, pedometers, and other tracking devices allow evaluation of the use of winter garden. Icken et al. (2011) studied exploratory behavior of Rhode Island Red hens between 9 and 16 months of age. Heritability of the duration of stay in the winter garden ranged from 20 to 30% and the frequency of passages between the garden and the building ranged from 30 to 50%. Both of these traits were highly genetically correlated with each other, meaning that birds that went outside more frequently also spent more time there. However, an unfavorable genetic correlation was estimated between the length of stay in the garden and the number of eggs laid in the nest, which suggests that hens that enjoyed being outdoors didn't bother to go inside to search for nests to lay eggs. There was also some detrimental effect on shell color.

In cage-free systems without beak treatment, selection to decrease feather pecking and cannibalism is also important. In addition to group selection, beak length has been suggested as a criterion to decrease feather pecking. This trait has a heritability of around 20% and is positively, though not very strongly, genetically correlated with mortality due to pecking. In some lines, beak length also has some correlation with plumage condition (Icken et al., 2017). Alemu et al. (2016) showed that selection for livability can be further improved by incorporation of genomic information (greater than 30% increase in accuracy of EBV over pedigree).

With the move to longer production cycles without molting, bone strength is also a concern (Preisinger, 2018). Bishop et al. (2000) created a bone index that combined tibial strength, humeral strength, and radiographic density of the keel bone. The heritability of the index was estimated at 40% and the index responded well to selection, with divergently selected lines differing by 25% in tibial strength, 13% in humeral strength, and 19% and keel density. The line selected for high bone strength also had fewer bone fractures. Andersson et al. (2017) focused on keel bone deformity evaluated by palpation. Estimates of heritability were ranged from 15 to 30%. Keel bone deformity did not have significant genetic correlations with persistency of egg production, egg weight, or shell strength but did have a negative genetic correlation with early egg production. This suggests that selective breeding can be used to reduce susceptibility to keel bone deformities but that this may be at the expense of early egg production.

Selection for crossbred performance

In addition to GxE, an additional consideration in modern poultry breeding programs is that poultry used for commercial production are typically crossbred, often a cross between four pure lines, but that most selection takes place in the pure lines (Figure 1). Crossbreeding invokes another source of interaction, i.e. interaction of the genetics of one pure line with the genetics of other pure lines in the crossbred product, i.e. GxG. In this paper, these interactions will not be considered explicitly but can contribute to a less than unit genetic correlation between phenotypes measured on purebreds in a nucleus environment and phenotypes on crossbreds measured in a commercial environment (Wientjes and Calus, 2017).

Poultry breeders were early adopters of the use of crossbreed data for breeding through systems such as reciprocal recurrent selection (Comstock et al., 1949), later developed into sib testing. This also assured that crossbred daughters of elite males had consistently high performance across different production systems and environments (Preisinger and Flock, 2000; O´Sullivan et al., 2010). In practice, selection for crossbred performance is typically implemented as combined crossbred-purebred selection (CCPS) (Wei and van der Werf, 1994). In CCPS, phenotypes collected on purebreds in the nucleus are combined with phenotypes collected on crossbreds in the field and used for genetic evaluation of purebred selection candidates for crossbred performance in the field, considering purebred and crossbred performance as separate but correlated traits.

Cavero et al. (2010) estimated genetic correlations between purebred birds kept in single-bird cages and crossbred performance in multi-bird cages and found that the estimates were moderate to high at sexual maturity (0.63 and 0.83), but the correlations decreased to moderate to low levels in the other two stages of production (between 0.1 and 0.5). In contrast, genetic correlations were high for egg quality traits such as egg weight, shell strength, and shell color (0.83 to 0.93 based on cage averages and 0.73 to 0.90 when based on individual records).

Strategies to deal with GxE in layer breeding programs

For an international breeding company, the most desirable strategy is to meet the diverse needs of a global industry by the smallest number of purebred lines. Since the final commercial product typically is a cross between 2 and up to 4 lines (see Figure 1), selection in the pure lines can target different breeding goals for each line. This allows the needs of some markets to be met through different crosses between the available pure lines (Leenstra et al., 2016). In other cases, additional selection at the multiplier levels can bring the commercial product closer to the needs of a specific market (Leenstra et al., 2016), noting that this selection does not result in changes in genetic gain in the pure line and does, therefore, not have a permanent and cumulative impact on performance.

If the same set of pure lines is used as the basis to provide crossbred birds across a range multiple production systems or markets, it is important that the needs of each of these markets are accounted for in the selection program, weighted by the relative importance of each market for the breeding company. This includes consideration of specific traits for specific markets and of the presence of GxE for other traits. For traits for which there is considerable GxE between the purebred nucleus and the commercial environment, it is important that phenotypes that are collected in that environment are used to inform selection decisions on the purebred selection candidates. If, in addition, considerable GxE exists between markets, phenotypes may need to be collected in multiple environments. If differences between environments can be quantified (e.g. climate related factors such as ambient temperature and humidity), reaction norms can be used to model GxE on phenotypes across a range of environments. Dekkers (2022) showed how reaction norm models can be used to optimize the environment in which phenotypes should be collected in order to maximize their value for a breeding program that targets a specific or range of commercial environments.

Selection for robustness and resilience

Robustness is defined as the capacity of an animal to maintain productivity in a wide range of environments without compromising reproduction, health, and wellbeing (Colditz and Hine, 2016). As described by Leenstra et al. (2016), breeding companies have started breeding for more robust birds by incorporating selection for additional traits such as docility, feather pecking, and nesting behavior, along with egg production and persistency.

Some environmental differences between production environments are caused by the presence of short-term disturbances, such as short-term heat stress, social disturbances, and disease, which can have a higher prevalence in some production environments than in others. To address such short-term environmental effects, in contrast to the systematic environmental or production system differences that are addressed by the concept of robustness, resilience is defined as the ability of an animal to be minimally affected by short-term disturbances or to rapidly return to the state before exposure to a disturbance (Colditz and Hine, 2016). For phenotypes that are recorded on a longitudinal basis over time, such as egg production, body weight, milk production, and feed intake, resilience can be quantified based on the distribution of an animal's phenotypes over time, deviated from some expectation, i.e. measures based on the distribution of residuals from a fitted model (Berghof et al. 2019a). This includes day-to-day variation in residuals (animals with larger variation are less resilient), skewness of residuals (animals with more negative residuals for, e.g., feed intake, are less resilient), the auto-correlation of residuals over time (animals with a negative autocorrelation of residuals are less resilient) (Berghof et al., 2019a), or the proportion of residuals that are considered negative outliers (Putz et al., 2019). These measures have been found to be low to moderately heritable and genetically correlated with disease incidence and veterinary treatment rates under a polygenic natural disease challenge in grow-finish pigs (Putz et al., 2019, Cheng et al., 2021). Similar measures of resilience were investigated in layers based on four-weekly body weights by Berghof et al. (2019b) and based on daily egg production by Bedere et al. (2022). The latter found that these measures were lowly heritable in both purebred birds housed in single-bird cages and in crossbreds housed in multi-bird cages, but were positively correlated with each other and with egg production. Further research is needed to determine whether these measures are related to resilience to disease and heat stress under different environmental conditions. Interestingly, in a preliminary study, Harlizius et al. (2020) found that boars that had higher day-to-day variation in feed intake in a high-health pig nucleus herd had progeny that had higher death rates in a disease challenge. This suggest that even measures of resilience to disturbances such as social, metabolic, and environmental stressors, which is what boars can experience in a nucleus environment, rather than disease, are genetically correlated to resilience to disease.

Development of specialized lines

An alternative to using the same set of lines to target a range of production systems, environments, and markets is to develop specialized lines. In some cases, e.g. for white versus brown egg markets, different pure lines are required because of the genetic nature of the brown egg shell color trait (determined by a dominant gene that is epistatic to the recessive white shell color gene (Punnett and Bailey, 1920), although some of the lines that contribute to commercial hens that produce white versus brown eggs can be common to both products. Requirements for egg weight can also differ substantially between markets by country and may require different pure lines. Egg weight is a polygenic trait with moderate to high heritability and different line crosses are used for markets that require small versus large eggs. When using (a) specialized line(s) for a specific market, it is still essential that phenotypes that are used for selection within the pure lines are collected in the right environment when substantial GxE is present between the nucleus and commercial environments.

Development and selection of specialized lines requires resources to be allocated to those lines. If breeding program resources are limited, e.g. in terms of the total number of purebred birds across lines, that can be produced, evaluated, and selected, or in terms of the amount of phenotypic data that can be collected in the field, every additional line removes resources from existing pure lines, which can affect the rate of genetic improvement achieved in these lines. Thus, modeling response to selection as a function of available resources is required to conduct a proper cost-benefit analysis when considering addition of specialized lines.

With single-trait selection, the rate of genetic improvement per generation in a pure line is a function of the product of the accuracy of selection, selection intensity, and the genetic standard deviation of the trait among the purebred selection candidates (Falconer and MacKay, 1996). This concept also applies to multiple-trait selection for an overall breeding goal H (Hazel, 1943), ($H = v_1g_1 + v_2g_2 + \ldots$, where g_i and v_i are the breeding value and economic value for trait i), by considering the breeding goal as a single trait. In this case, accuracy of selection is the accuracy of the total merit index as a predictor of a purebred selection candidate’s true value for the breeding goal H, and the genetic standard deviation is the genetic standard deviation of the breeding goal among selection candidates (Dekkers and Gibson, 1998). When limited breeding program resources need to be divided among more pure lines, this can impact response to selection for existing pure lines by reducing all three factors that contribute to genetic gain, as will be illustrated in the example that follows.

Design of a global layer breeding program and the impact of genomics

To illustrate decisions on a combined versus specialized breeding programs and the impact of availability of genomic predictions on these decisions, an example with selection for a breeding goal consisting of egg production and egg weight was investigated, as illustrated in Figure 2. Three production environments were considered: 1) a single-bird cage system, representing a typical high-health nucleus breeding environment (nucleus), 2) a multi-bird cage production system, and 3) a floor production system. Assumed genetic parameters are in table 1. Note the low genetic correlations between the three production systems for egg production (0.5 and 0.2 between the nucleus and the multi-cage and floor systems, respectively, and 0.5 between the two field production systems). For egg weight, genetic correlations among the three production systems were set to 0.8. Accuracies of genomic prediction for each trait in each environment were derived using the method of Dekkers et al. (2021) based on a training population of 10,000, an effective number of chromosome segments (Me) equal to 3705, as derived in Dekkers et al. (2021) based on data from the genomic selection experiment in layer chickens described by Wolc et al. (2015), and a genotyping panel of 40,000 markers.

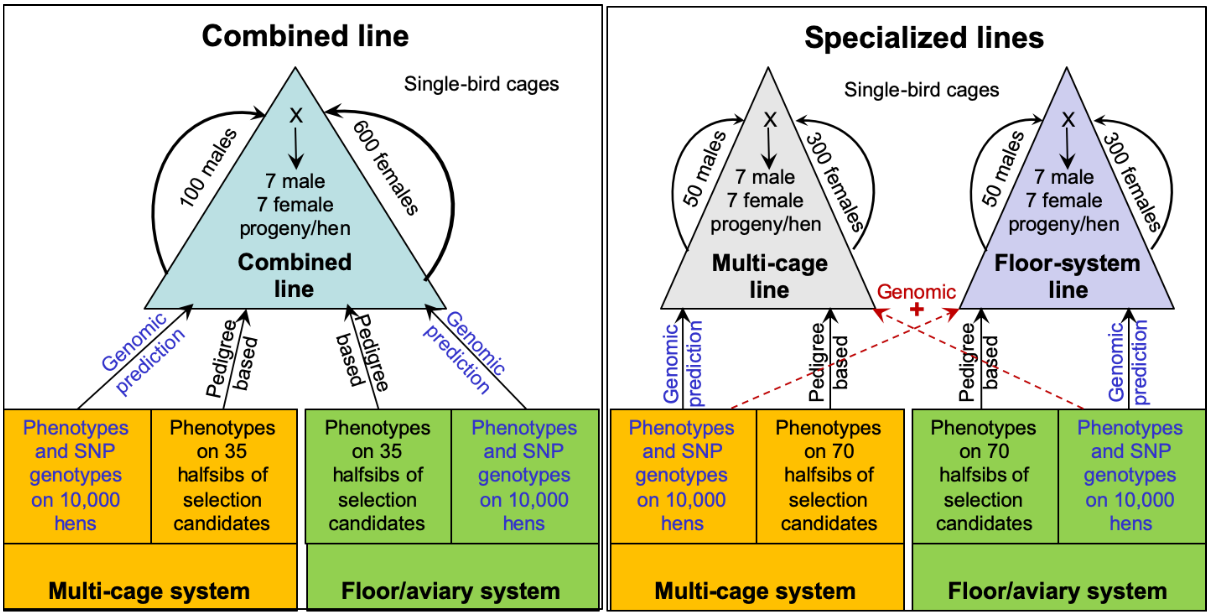


Figure 2. Schematic representation of the breeding programs simulated.

Table 1. Genetic parameters used for the example.

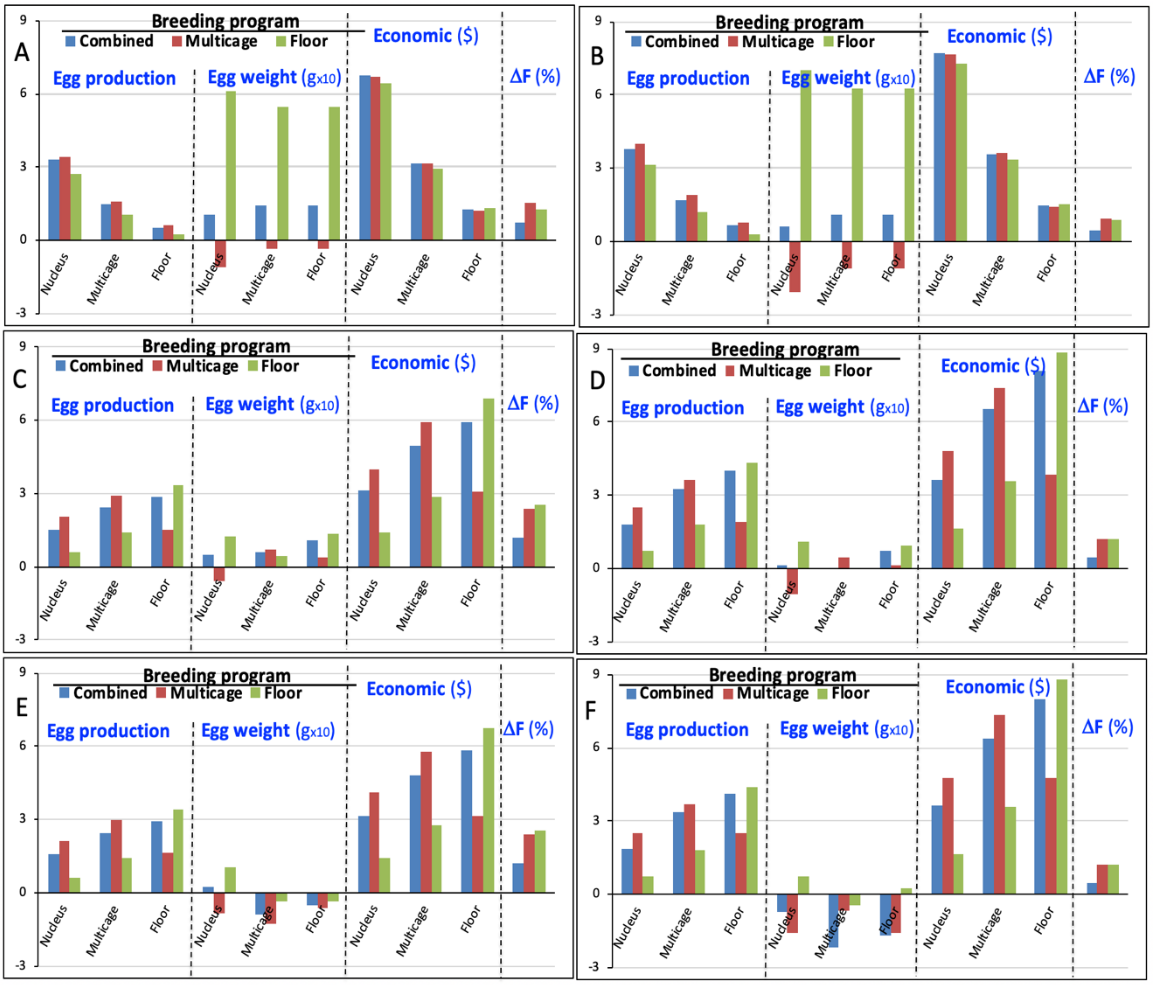
Environment	Trait	Genetic (above diagonal) and phenotypic correlations						Heritability	Phenotypic SD	Genomic Prediction Accuracy
		Nucleus		Multi-cage		Floor				
		Prod.	Weight	Prod.	Weight	Prod.	Weight			
Nucleus	Prod.		-0.35	0.50	-0.25	0.20	-0.25	0.20	10.0	0.54
	Weight	-0.20		-0.30	0.80	-0.20	0.80	0.55	2.8	0.76
Multi-cage	Prod.				-0.30	0.50	-0.25	0.17	11.0	0.51
	Weight			-0.20		-0.25	0.80	0.50	3.0	0.74
Floor	Prod.						-0.25	0.20	11.8	0.54
	Weight					-0.20		0.50	3.0	0.74

The nucleus breeding program consisted of 100 males and 600 females selected each generation, with each female producing 7 females that were recorded for egg production and egg weight in single-bird cages (Figure 2). The target production systems for the breeding program were multi-cage and floor (aviary) production systems. For the combined breeding program, selection in the nucleus population was for a breeding goal that consisted of egg production and egg weight in both target production systems, with weights of 2 and 1.5 on egg production and egg weight, respectively, in each production system. Thus, the markets for the two target production systems were assumed to be of equal size. For the breeding program with specialized lines, the nucleus population was split in two, with one half (50 males and 300 females each generation) selected for the multi-bird cage production system and the other half selected for the floor production system (Figure 2). Thus, the total size of the nucleus and the amount of recording in the nucleus was kept constant. Asymptotic responses to selection and rates of inbreeding were computed using the deterministic software SelAction (Rutten et al., 2002), with genomic predictions incorporated as described by Dekkers (2007), based on the accuracies in Table 1.

Responses to selection in each production system (including the nucleus) from the combined and specialized breeding programs are in Figure 3. Note that these are asymptotic responses that do not account for the reduction of genetic variance due to inbreeding (Rutten et al., 2002). Figures 3A and B show responses to breeding programs when phenotypes were only recorded in the nucleus for, respectively, pedigree-based and genomic selection breeding programs. For these programs, responses in egg production were greatest in the nucleus, as expected, because phenotypes were recorded in the nucleus, and lowest for the floor system because the assumed genetic correlation between production in the nucleus and in the floor system was only 0.2. Responses for egg weight were very variable across the three systems, as a result of the assumed genetic parameters. When the floor system was the target, egg weight increased substantially because selection for production was ineffective due to the 0.2 genetic correlation between production in the floor system and in the nucleus, where phenotypes were recorded. In economic terms, responses for the breeding goal in a given environment can be computed by summing responses for production and egg weight, multiplied by their economic values (2 and 1.5). Economic response was greatest for the nucleus, about half that for the multi-cage system, and 5 times as small for the floor system.

Splitting the population into specialized lines resulted in similar economic responses in the target environment (multi-cage or floor) as the combined breeding program when phenotypes were only collected in the nucleus (Figures 3A and B). However, the rate of inbreeding roughly doubled when lines were split because of the smaller numbers of sires and dams used within each population (50 and 300) compared to the combined breeding program (100 and 600). The use of genomic prediction (Figure 3B) increased economic responses to selection by about 14% for each production system. However, responses were still substantially greater in the nucleus than in either the multi-cage or floor systems because the genomic predictions were also based on phenotypes recorded in the nucleus. Genomic prediction also reduced rates of inbreeding but they were still about twice as large for the specialized lines compared to the combined breeding program.

Figure 3. Rates of inbreeding (ΔF) and asymptotic responses to selection per generation for egg production and egg weight (0.1 gram units) and economics ($) in the nucleus, multi-cage, and floor production systems (x-axis) from a combined breeding program (🟦) and from specialized breeding programs for multi-cage (🟥) and floor production (🟩), with phenotype recording in the nucleus only (**A** and **B**) and in the nucleus and in the field (**C** and **D**: production and egg weight recorded; **E** and **F**: production only recorded in the field) and without (pedigree-based selection; left plots) and with (genomic selection; right plots) availability of genomic predictions.



The low responses in the multi-cage and floor systems can be addressed by recording phenotypes in those environments, either through relatives or genomic prediction. To investigate this in a pedigree-based system, a total of 3,500 female paternal half-sibs of selection candidates were assumed to be recorded for egg production and egg weight in both the multi-cage and the floor system and used for pedigree-based genetic evaluation. So in the combined program, each selection candidate had 35 paternal halfsibs with records in the multi-cage system and 35 in the floor system (Figure 2). In the specialized lines, each selection candidate in the multi-cage line had 70 paternal halfsibs in the multi-cage environment and each selection candidate in the floor line had

70 paternal halfsibs in the floor environment, for a total of 70x50=3,500 birds recorded in each field environment. Although collecting egg production and weight data on individual birds in the field is problematic, especially in multi-bird cage systems, for simplicity, it was assumed that this was possible in both systems. With genomic selection, it was assumed that a training data set with phenotypes and SNP panel genotypes on 10,000 birds was available for each production system (Figure 2). In the specialized lines, only phenotypes recorded in the nucleus and in the target environment (multi-cage or floor) were used for genetic evaluation and genomic prediction, as reflected in Figure 2. Thus, the floor system line used phenotypes and genomic predictions for production and egg weight recorded in the nucleus and in the floor system, while genomic predictions for traits recorded in the multi-cage nucleus line and in the multi-cage environment were not used for genetic evaluation in the floor system line.

Figure 3C shows responses using pedigree-based selection with egg production and egg weight phenotypes recorded in the field. Compared to selection using phenotypes collected in the nucleus only, adding field records substantially increased response for production in the field environments, while it substantially reduced production response in the nucleus. Responses in egg weight were small positive and much more consistent across the different production systems. Economic responses in the field environments were also substantially higher when field records were included for genetic evaluation. However, the use of these phenotypes also more than doubled rates of inbreeding because field records are on half-sibs of the selection candidates, which increases emphasis on family information. The addition of genomic predictions from both the nucleus and field environments (Figure 3D) resulted in substantially greater responses in production in the targeted field environments. Economic responses also were substantially greater and rates of inbreeding were substantially lower.

Table 2 summarizes the additional economic responses within the target environment (multi-cage or floor) when using specialized lines versus a combined breeding program. Compared to the combined selection program, specialized lines realized greater economic response for their respective environments. Extra responses from the use of specialized lines were substantially greater without than with genomic prediction, both in absolute and relative terms: 19.8 and 16.3% for the multi-cage and floor system lines, respectively, without genomics, compared to 12.7 and 8.9% with genomics. This suggests that the use of specialized lines is less beneficial with the use of genomic selection. This is likely because the combined program benefits from genomic information from both field environments, compared to the use of only genomic predictions from its target environment for each specialized line. In principle, however, genomic information from the floor environment could also be used as a source of information for the multi-cage selection line, as illustrated by the Genomics+ scenario in Figure 2, although the relevance of that information would decline as the two lines diverge from each other over generations (Wientjes et al. 2017). Under the assumption that there’s no decline in relevance, the Genomics+ scenarios in Table 2 show extra responses when training data from the other environment were also used for genetic evaluation of the specialized lines. This increased the extra responses but, on a relative scale, still not to the level observed for the pedigree-based breeding program.

Table 2. Extra economic response (asymptotic) from selection using specialized lines compared to the combined breeding program in absolute ($) and relative (%) terms for pedigree-based and genomic selection programs. Genomic+ refers to the case when training data from both field populations were used for genomic prediction in the specialized lines, assuming no decline in relevance of the training data from the other line as the lines diverged.

Phenotype recording	Genetic evaluation	Multi-cage		Floor	
		$	%	$	%
Nucleus only	Pedigree	$0.00	0.0%	$0.04	2.9%
	Genomics	$0.01	0.4%	$0.05	3.5%
Nucleus + Field Production + Weight	Pedigree	$0.98	19.8%	$0.96	16.3%
	Genomics	$0.83	12.7%	$0.72	8.9%
	Genomics+	$1.03	15.7%	$0.91	11.2%
Nucleus + Field Production	Pedigree	$0.96	19.9%	$0.93	15.9%
	Genomics	$0.95	14.8%	$0.79	9.9%
	Genomics+	$1.00	15.6%	$0.88	10.9%

Because genetic correlations between productions systems are relatively high for egg weight, one could save costs by recording only egg production data in the field. To investigate this, Figures 3E and F show responses when egg weight data are only recorded in the nucleus. Dropping these data in the field had limited impacts on responses in production, both without and with genomics, but resulted in negative responses for egg weight in the field environments. This resulted in small reductions in economic responses compared to the situation when egg weight was also recorded in the field (Figures 3C and D). However, the extra responses from the use of specialized lines compared to a combined breeding program were little affected by dropping egg weight recording in the field. Thus, for this example, the establishment of specialized lines was beneficial, at least in the short term. In the longer term, however, the higher rates of inbreeding incurred within the specialized lines will reduce genetic variance and response to selection.

In strategy Genomics+, genomic information is exchanged between the two specialized lines. This increases response to selection and the benefit of specialized lines over a combined program. The benefit of exchanging genomic information between environments will be especially beneficial for populations/environments with small training data, as demonstrated for dairy cattle by Slagboom et al. (2019). Over time, as the lines diverge, the contribution of training data from the other environment and population will, however, diminish.

In addition to exchanging genomic information, it is also possible to exchange genetics, by selecting the best animals for each environment from across the specialized lines.

This exchange of genetics would also help to reduce the rate of inbreeding within each line. The value of this strategy was also demonstrated by Slagboom et al. (2019). Selection across lines is, however, only effective and beneficial if the genetic correlation between the breeding goals of the two lines is not too low and the lines have not diverged too much.

Slagboom et al. (2019) investigated the break-even correlation for genomic selection for two target environments in dairy cattle, which is the genetic correlation between the breeding goals for the two environments below which establishment of two specialized lines results in greater responses to selection then the use of a combined program. For a genomic selection program with exchange of both genomic and genetic information (i.e. across-line selection), they found the break-even correlation to be equal to 0.65, which was not different from the break-even correlation obtained by Mulder et al. (2006) for a pedigree-based progeny testing program. Thus, the availability of genomic predictions did not affect the break-even correlation. The break-even correlation was also not affected by restricting selection to only within line, although that did reduce response to selection compared to across-line selection. However, also restricting the use of genomic information to within lines, reduced the break-even correlation to 0.57 and also reduced genetic gain. Thus, without exchange of genetics or genomics between lines, the establishment of specialized lines is only beneficial when the genetic correlation between the breeding goals is low. In the layer example investigated here, the crucial genetic correlation is that between the multi-cage and floor environments, which was 0.5 for production and 0.8 for egg weight. For those correlations, specialized lines resulted in greater asymptotic responses to selection. However, the larger rates of inbreeding with the use of specialized lines would be a major concern and additional resources to enlarge the breeding populations may be needed to make the use of specialized lines beneficial in the longer term.

Conclusions

Diversification of production systems for layers creates significant GxE interactions in terms of individuals traits (in particular for egg production and livability) and breeding goals (relative importance of traits). This can be addressed by development of specialized lines or line combinations that are optimized for the target environment or by breeding lines that perform well across environments by broadening the breeding goal to include all traits that are relevant across the environments and breeding for robust animals that are adaptable to different environments. Collecting phenotypes in the target environments and using genomic predictions can increase genetic gain in either scenario. Genomic selection offers opportunities to utilize data collected on other lines and in other environments for genetic improvement of a given line. The optimal breeding scheme is conditional on genetic correlations between the environments, population size (risk of inbreeding in case of small, specialized lines), and the cost of both phenotyping and genotyping, and must be evaluated on a case by case basis.

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How far can we go with enhancing the environmental sustainability of poultry systems? (I. Kyriazakis)

Ilias KYRIAZAKIS

Institute for Global Food Security, School of Biological Sciences, Queen’s University, Belfast BT9 5DL, United Kingdom

i.kyriazakis@qub.ac.uk

Broiler and layer systems are the least environmentally impacting amongst livestock systems, as far as their global warming potential emissions are concerned (C footprint). The question addressed in this presentation is how far can we go to reduce further their environmental impact, especially in the case of broilers which have already the lowest C footprint amongst livestock systems. I consider three strategies to address this: 1) Can we reduce further the environmental impact of poultry systems by relying more on home-grown protein sources? In Europe heavy reliance on imported soya is considered as the main contributor to their environmental impact. 2) Can we reduce their environmental impact by including alternative protein sources that rely on novel growing methods and biotechnological advances? 3) What will happen to the environmental impact of broiler systems if we switch to slower growing birds, which is the current tendency in Europe?

Detailed answers to these questions will be presented but a brief summary is given here: (i) Reliance on home-grown protein sources will enhance the environmental impact of both broiler and layer systems only when they replace non-sustainable soya in their diet. (ii) Not all alternative protein sources will reduce the environmental impact of poultry systems. This will depend on the impacts associated with their production. For example, insects whose production is associated with drying inputs will not reduce the environmental impact of poultry systems. (iii) By definition, breeding for slower growing and/or less efficient birds will increase the environmental impact of broiler systems. Feeding these birds on alternative protein sources will confer less reductions in the environmental impacts of their systems, than when feeding them to faster growing birds. Poultry systems are already at an advantage in terms of the C footprint compared to other livestock systems. Whether this environmental impact can be reduced further will depend on the interplay of what birds we will be using in the future and the protein sources they will be given access to.

PS5

New frontiers in poultry science

Dominic WRIGHT

Authors Martin-Cerezo, M-L., Johnsson, M., Bakovic, V., Gering, E., Henriksen, R., Wright, D.
E-mail address dominic.wright@liu.se
Abbreviated title Domestication, Feralisation and Adaptation

Summary

Feralisation is the process by which domestic animals are returned to the wild, often going on to hybridise with their wild counterparts or carve out novel niches independently. This can be a highly effective model of natural selection, coming from a known genetic starting point, and depending on the organism in question, an important reservoir of beneficial gene diversity. I will present ongoing work based on a sample of 469 feral birds sampled from the islands of Bermuda and Hawaii. These birds have all been sequenced to at least a 10X depth, resulting in over 24 million SNPs identified in the two populations. In addition, four different tissues were sampled (hypothalamus, cerebellum, femoral bone, comb tissue) and transcriptomes generated from a total of over 1500 samples across these tissue types. These animals were phenotyped for comb mass, weight, anxiety (anti-predator) behaviour, bone density, parasite-load and other life history traits, with these then being whole genome mapped to identify the genetic loci underpinning these traits. By combining selective sweep mapping, eQTL and QTL from these different tissues and traits I aim to demonstrate the genomic regions and traits which are under selection in these populations and the potential pleiotropy that exists at these loci in terms of gene and trait regulation. By combining these results together, functional genes and even mutations can be identified that influence a wide variety of traits.

Keywords Feralisation, Domestication, QTL, eQTL, Genetical Genomics,

Feralisation, the process that occurs when domestic animals return to a wild environment, can be thought of as domestication in reverse. Whereas domestication has been both the cornerstone of civilization, and an area of intense study to help understand evolution and how genes can affect phenotype, feralisation remains less well-studied. Under feral conditions the natural and sexual selection pressures of the wild are reintroduced, while artificial as well as natural selection for the captive environment are abolished.

Individuals will once again be subjected to mate choice decisions by the opposite sex, and will undergo selection based on the number of offsprings they have, degree of parental care they provide, and other evolutionary trade-offs based on resource allocation that they will not have been subjected to in the domesticated habitat. Evolutionary responses to these selection pressures will depend on both the genetic variability of feral populations and the genetic architecture of fitness-related traits[2,3].

These properties of feral populations result from combined histories of domestication and feralisation, each of which can be complex[4-8]. By characterizing genetic and phenotypic variation in feral taxa, the genomic study of feralisation can answer similar questions to domestication, only from a different perspective, principally the changes driven by natural environmental conditions. Feralisation can therefore give tremendous insights into how a genome that is suited to a highly specialised and controlled environment is shaped under natural conditions, and what genes respond. Particularly, sexual selection and returning mate choice, can have massive effects on such a population, and one can therefore identify genes and alleles that are relevant to sexual selection and mate choice, a vital area of study in evolutionary theory[9]. Similarly, feral animals can also be thought of as an invasive species and shed light on concerns of intense interest such as how adaptation can occur, how rapidly this happens, as well as how phenotypic diversity can be generated from a small founder population. Feral populations often display high population variability (in both genetic and phenotypic terms) despite typically coming from limited founder populations, and therefore provide a strong model to study how cryptic variation can be revealed. Despite such potential benefits, current research on feralisation has been largely limited to phenotypic assays, with few studies having examined how this process shapes feral gene pools and the genes underlying the process. Genetic studies using molecular techniques have been limited to genetic markers to broadly assess the degree of introgression between wild and feral species[10] (generally how many domestic genes are present in wild populations) and the overall population structure, with studies primarily on pigs[11], wolves[8,12]and wild cats. Currently almost nothing is known about what loci will be affected and how extreme such changes may be. Are these loci the same or the reverse of those selected by domestication? The feral genetic model has relevance to natural populations, whilst the classic domestication model has a wealth of genetic and genomic resources in addition to the knowledge of how selection has shaped its phenotype. The combination of the two will allow the identification of the causal genes and even polymorphisms and mechanisms of gene action that are responsible for quantitative variation in a range of organismal traits in a feral population, with this a central goal in almost all genome-wide association studies performed in natural populations (most notably humans).

The feral chickens on Kauai and other Hawaiian Islands are a valuable resource to study the effects of feralisation and the gene targets of selection. In terms of their origin, archaeological evidence indicates that chickens were first introduced to the Hawaiian Island chain (including Kauai) by AD1200 via human migration into the eastern Pacific[13,14]. Their sources were most likely Red Junglefowl (RJF – *Gallus gallus*) transported from the western Pacific by Polynesian settlers[13]. Therefore, wild RJF have likely persisted on these islands for over 1000 years. In the 1980s, tropical storm Ewa and Hurricane Iniki destroyed many of the coops containing Kauai’s domestic chickens, releasing their occupants into local forests, and spurred large-scale species invasions. Our mitochondrial, vocalisation and plumage-based analyses concur, indicating that this population is a hybrid with both domestic and pacific origins[15]. Birds inhabiting Kauai today exhibit characteristics of both the original RJF founder strain and more recently derived European domestics, and these characteristics may be involved in adaptation to feral environments. What makes the Kauai population even more valuable is the presence of other feral chicken populations on the neighbouring islands (Oahu, Maui and Hawaii). These islands are rigorously kept isolated from animal transfer, meaning that they are perfect independent replicates of feralisation. As such, the chickens on Kauai represent an incredibly valuable resource for conservation and scientific study, allowing examination of the causes and consequences of admixture and feralisation, as well as the genetic and phenotypic evolution associated with feralisation.

This project presented here uses the twin aspects of feralisation and domestication to disentangle the genetic effects of sexual selection and natural selection, looking at traits ranging from comb size, brain size, behaviour, and bone allocation. Evolutionary theory and previous data indicate that feralisation may be acting strongly on sexual ornamentation, plumage and related fecundity traits. We have shown that the vocalisations and plumage in these feral birds display high variability[1], while comb size also shows strong differences to domestic birds. Given the potential bottleneck in the starting population, the apparent extreme population variability for many of these traits is even more remarkable. Previous work by myself and others has shown that a number of potential trade-offs arise from the sexual ornament of the chicken[2-5], with increased comb size reflecting greater investment in egg production and a decrease in female medullary bone density (calcium either being used to strengthen the bones or for the production of eggs). Notably, this trait is an example of a female as well as male sexual ornament. A theory of sexual selection proposes that the ornament transmits reproductive or fitness information to the receiver[6], with the comb of the chicken therefore being an honest indicator of quality and fitness, with genetic links between these characteristics. Understanding the relationship between calcium availability, bone density, egg production and comb mass in the wild will be a direct test of this ornament in the face of genetic and environmental limitations and resource allocation issues, and will answer how and if a sexual ornament persists with changing selection pressures[7]. The results will also allow us to identify novel loci that can affect bone strength and fracture resistance, which are vital for layer chicken breeds in particular.

Brain size and composition is assumed to underpin cognitive and behavioural phenotypes. Trade-offs are thought to exist between the costs of developing and maintaining energetically expensive tissue and specific physiological[8-10] and behavioural[11,12] variables. In particular the cerebellum and cerebral hemispheres have been linked with foraging ecology[12] and social environment[13]. We have previously shown that proportional cerebellum size is correlated with brooding/ maternal behaviour (as well as explorative tendency) in the chicken[14]. The Kauai chickens have very high variability in proportional cerebellum size, enabling these correlations and trade-offs to be tested in a wild population. In the current work, I will present how we can identify genes responsible for regulating intra-population variation in the mass of brain sub-structures, and ascertain if potential pleiotropy exists.

Selective sweep mapping is feasible in the Kauai chicken, as demonstrated by our previous study[15], that identified multiple sweeps, as well as the origin of each sweep (principally domestic, with some RJF). Whole genome chromosomal painting indicated that the population is extremely hetergenous, though well mixed, with no strong population substructure. For this analysis, different islands in the Hawaiian island chain have been compared (Kauai, Oahu, Hawaii), as well as an additional population from Bermuda, which acts as an out-group of feralisation. The Bermudan population has no RJF founder individuals and is entirely comprised of various domestic birds that were released, making it ideal for this task. All individuals were sequenced to 10X, with 24 million SNPs identified. A number of sweeps were detected, including several that were present in both Hawaii and Bermudan, indicating that certain regions appear to be commonly selected upon in different and diverse feral populations.

To actually identify the causal variants and gene polymorphisms that exist in these feral populations, a combination of genome-wide mapping with expression genome wide association analysis (EWAS) is used. Regions containing causal variants for transcript abundance are overlaid with those affecting organismal traits, before genes are assessed with causation modelling for putative effects on the traits of interest. Using this technique, I will present results from behavioural, morphological and life-history traits, in combination with transcriptomic data from hypothalamus, cerebellum, femoral bone, and comb base tissue, to demonstrate how causal genes can be identified. Finally, these are overlaid with the sweep regions to identify those traits that are the focus of selection during the feralization process.

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Jae Yong HAN

Jae Yong Han* and Jin Se Park

Biomodulation major, Department of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea

*Corresponding author: jaehan@snu.ac.kr

Summary

Primordial germ cells (PGCs) are the most promising germline competent stem cell for production of avian germline chimera because they can be easily isolated and long-term cultured *in vitro*, and migrate to embryonic gonads when injected into embryonic blood vessels. The development of programmable genome editing technologies such as TALEN and CRISPR/Cas9 system, and the successful combination of these technologies with PGC culture systems, has allowed the production of various genome edited chicken models. Genome editing in cultured chicken PGCs facilitates the production of highly valuable chicken models for 7 various industrial applications, including production of disease resistant aves, avian bioreactor systems, sexing at the embryonic stage, and egg white protein component modification. Furthermore, as a valuable model animal for developmental biology and biomedicine, genome editing can be used to produce chickens with gene knockouts that can elucidate gene function, or with a reporter gene inserted into a specific gene locus to produce a cell tracing system. Overall, genome editing in chickens has the potential to greatly benefit both the biotechnology industry and academics. In the near future, a variety of genome edited chicken models that can be applied to industry will emerge.

Keywords: Avian research model, Industrial application, Genome editing, Primordial germ cell

Introduction

Chickens are valuable model animals for humans in both the food industry and biomedical sciences. They serve as an abundant source of proteins by providing meat and eggs, and chicken eggs are widely used as a major ingredient for food processing industries. Furthermore, in the pharmaceutical industry, chicken eggs serve as a vaccine production platform and bioreactor for therapeutic enzyme generation. Finally, chickens have been used as experimental model animals to study developmental biology and immunology (Stern, 2005). The application of genome editing technologies to chickens will rapidly enhance all of these beneficial characteristics. Therefore, research on the introduction of genome editing in chickens for development of valuable models has been conducted globally.

The most prominent germline competent cell for genome editing in chickens is the primordial germ cell (PGC). PGCs are early progenitor germ cells that can differentiate into functional gametes. Unlike mammals, chicken PGCs migrate to embryonic gonads through the bloodstream and can be efficiently isolated from embryonic blood and gonads. Furthermore, these isolated chicken PGCs can be long-term cultured *in vitro*, and efficiently produce germline chimera and genetically engineered progenies when transplanted into recipient embryos (van de Lavoir et al., 2006, Park and Han, 2012). The discovery that chicken PGCs can be maintained *in vitro* without loss of germline competency by activation of MEK/ERK and SMAD signaling pathways has resulted in firmly established chicken PGC culture methods (Choi et al., 2010, Whyte et al., 2015).

Following the development of programmable genome editing (PGE) technologies such as TALENs and CRISPR/Cas9, these techniques have been applied to cultured chicken PGCs, and several successful knock-in and knock-out studies have been reported (Park et al., 2014, Oishi et al., 2016, Dimitrov et al., 2016, Taylor et al., 2017). As a result of improved genome editing efficiency by PGC culture, various genome edited chickens that possess valuable traits have been produced in the last decade. In particular, genome editing in chickens has been performed to modulate egg components, confer disease resistance, and develop avian models for biological research. Several successful studies have been reported, especially in modulation of egg white components and acquisition of disease resistance. Using genome editing technology, egg white protein genes can be knocked-out, or foreign proteins can be efficiently accumulated at industrially meaningful levels in egg whites (Park et al., 2014, Oishi et al., 2018, Mukae et al., 2021). Furthermore, host factors recognized by avian leukosis viruses (ALV) for their entry into cells have been edited by CRISPR/Cas9 to produce genome edited chickens that are resistant to ALV infections (Koslova et al., 2020). These reports demonstrate that by using cultured PGCs, genome editing in chickens is now practically performed worldwide, and that valuable traits for industrial applications can be efficiently introduced into chickens.

Here, we briefly describe recent advances in chicken genome editing before discussing future perspectives for genome edited chickens in industry and in academic fields.

Current genome engineering in chickens using cultured PGCs

Following the establishment of techniques for long-term culture of chicken PGCs, it became possible to engineer PGCs *in vitro* and produce genome engineered progenies by transplanting engineered PGCs into recipient embryos and further mating. The first genome edited chickens were produced by HDR-mediated gene targeting in cultured PGCs (Schusser et al., 2013). In this study, immunoglobulin gene knockout chickens were produced, and these chickens showed defective B-cell development. However, the gene targeting efficiency of this approach was extremely low and thus impractical for wide application of genome editing in chickens. Following the development of PGE technologies that efficiently induce DSBs at target sites, knock-out and knock-in of cultured PGCs have been effectively performed, and various genome edited chickens have been produced. In the last decade, genome editing in chickens has been performed to modulate egg components, confer disease resistance, and develop avian models for biological research.

Genome editing for egg white component modulation

The main egg white proteins are ovalbumin (OVAL), ovotransferrin (TF), ovomucoid (OVM), and lysozyme (LYZ). These proteins are the major cause of egg allergy (Miller and Campbell, 1950, Hoffman, 1983), and thus, modulation of these egg white components by genome editing to produce hypoallergenic eggs would be highly beneficial. In 2014, using TALENs, PGE technology was first applied to cultured chicken PGCs to produce genome edited chickens in which the *OVAL* gene was knocked-out (Park et al., 2014). This research demonstrated that PGE technology can be practically applied to chickens to induce targeted genome editing. Subsequently, a premature stop codon was induced into the *TF* gene, using base editing technology, to produce genome edited chickens that laid eggs with reduced TF in their egg white, demonstrating that precise nucleotide changes at a target site can be efficiently performed in chicken PGCs (Lee et al., 2020). Recently, production of OVM deficient eggs was reported by applying CRISPR/Cas9 technology (Mukae et al., 2021). These studies show that egg white components can be modulated by genome editing technologies to allow development of hypoallergenic eggs.

Chicken eggs have been considered as efficient bioreactor for recombinant protein production because of hen’s high egg laying rate, high capacity to secrete proteins into eggs and relatively simple protein composition of egg white. Indeed, as proof-of-concept, it was demonstrated that functional human cytokines can be produced from egg white (Lillico et al., 2007, Herron et al., 2018). In addition, the N-glycosylation pattern of recombinant proteins derived from egg white proteins is characterized by abundant terminal mannose residues with lack of core fucosylation (Kim et al., 2018a). Based on these distinct glycosylation patterns, it was shown that monoclonal antibodies produced from egg white had significantly improved Fc effector functions, and that the chicken bioreactor system provides an optimal platform for production of anticancer antibodies with improved efficacy. Subsequently, CRISPR/Cas9 mediated genome editing was used to generate hens that produced functional anti-HER2 monoclonal antibodies in their eggs (Mukae et al., 2020). These studies demonstrate that functional biodrugs with improved efficacy can be produced using a chicken bioreactor system, and that production efficiency may also be significantly improved by genome editing.

Genome editing for acquiring disease resistance

Genome editing is an efficient tool to improve the economical traits of chickens, especially viral disease resistance. Viruses use cell surface molecules as “host factors" for entry into target cells and replication (Dimitrov, 2004). Therefore, disruption of viral host factor expression or precise editing of amino acid sequences critical for viral interaction can prevent viral infection. In the case of ALV subgroup J (ALV-J), tryptophan residue 38 (W38) of chicken Na^+/H^+ exchanger type 1 (chNHE1) protein is critical for interaction between the virus and chNHE1. Direct modification of W38 efficiently confers resistance to ALV-J infections in chicken fibroblasts (Lee et al., 2017a). Similarly, introduction of a premature stop codon in the cysteine rich domain of the tumor virus locus B (*tvb*) gene confers resistance to ALV-B infection (Lee et al., 2017b). Subsequently, sequential mutation of the *tva*, *tvb*, and *chNHE1* genes in chicken fibroblasts resulted in simultaneous resistance to ALV-A, B, and J infections (Lee et al., 2019a). Recently, genome edited chickens in which the W38 residue of chNHE1 is deleted have been produced and show resistance to ALV-J infection, without any additional phenotypic abnormalities (Koslova et al., 2020).

Chicken ANP32A protein is an important host factor that supports the viral polymerase (vPol) of avian influenza virus (AIV) in infected cells (Long et al., 2016). Furthermore, it was reported that Asp149 and Asp152 residues of ANP32A are involved in supporting vPol activity and precise editing of these residues showed significant reduction of viral replication in chicken cells (Park et al., 2021). These results suggested that AIV resistant chicken breeds can be established by precise editing of specific residues of chicken ANP32A using PGE technology.

Genome editing for development of research model animal

Chickens are a valuable research model for studying developmental and germ cell biology because access to developing embryos is easy, and PGCs possess a unique migration pattern. *DMRT1* gene knockout chickens have been established to allow elucidation of chicken sex determination mechanisms. Disruption of the *DMRT1* gene induces gonadal feminization in the male embryo, but functional female reproductive organs cannot be formed because of disturbed hormone synthesis (Lee et al., 2021). Another study showed that *DMRT1* gene disruption induces gonad feminization of male embryos and further demonstrated that the *DMRT1* gene determines gonadal sex by regulating estrogen synthesis (Ioannidis et al., 2021). Recently, to elucidate PGC formation and developmental processes, transgenic chicken germ cell tracing models, in which the green fluorescent protein (GFP) reporter gene was precisely inserted into the deleted in azoospermia like (*DAZL*) gene, have been developed (Rengaraj et al., 2022). Because the *DAZL* gene is a germ cell specific marker, GFP is specifically expressed in germ cells and thus can be used as a germ cell tracing model. Using this model, germ cells in all developmental stages can be isolated and provide resources for elucidating the transcriptome of germ cell populations at each developmental stage. To improve animal welfare, avian sexing models have also been developed by inserting the GFP reporter gene into the Z chromosome to allow efficient sex identification at early developmental stages (Lee et al., 2019b).

Genome editing for germ cell sterilization models

One drawback of PGC mediated genome editing is the variable germline transmission efficiency of cultured PGCs (Song et al., 2014). The efficient way for improving germline transmission efficiency of transplanted PGCs is to reduce endogenous germ cell population of recipient embryos. One method for reducing the endogenous germ cell population is treatment of recipients with busulfan; this method has been successfully used to produce germline chimera in poultry (Nakamura et al., 2008, Kim et al., 2018b). Recently, an *in vivo* donor germ cell enrichment model has been established in which busulfan resistant PGCs that overexpress the microsomal glutathione-S-transferase II (*MGSTII*) gene are transplanted. Using this system, busulfan injection into germline chimera results in specific depletion of the endogenous germ cell population in testes and thus significantly improves production efficiency of transgenic progenies (Kim et al., 2021). Also, an inducible germ cell sterilization chicken model, in which an inducible caspase-9 (iCaspase9) gene is inserted into the *DAZL* gene, has been developed, and endogenous germ cells were efficiently depleted in this system by injection of the chemical compound that promote dimerization of iCaspase9 and subsequent apoptotic pathway (Ballantyne et al., 2021).

Future perspectives and conclusion

The advent of PGE technologies, especially CRISPR/Cas9, and application of these technologies to cultured chicken PGCs have allowed the rapid development of genome edited chickens in recent years. Using precise genome editing, valuable economical traits, such as disease resistance, have been successfully introduced without any foreign gene integration. These technologies will be expanded to develop various disease resistant chicken lines by precise editing of host factors. Furthermore, when combined with molecular breeding technologies, genome editing can improve the quality of chicken meat and eggs in terms of taste and nutritional value. Meanwhile, by modulating egg components, genome editing will contribute to establishment of efficient bioreactor platforms for economical production of various therapeutic proteins with enhanced efficacy. Based on the beneficial N-glycosylation pattern of egg white proteins, various anticancer monoclonal antibodies with improved efficacy can be actively produced, and this will contribute to reducing the cost of these expensive biodrugs. Efficient genome editing in chickens will also further accelerate the development of various avian research models, such as a novel avian immune-deficiency model, for basic immunological research.

Collectively, genome editing in chickens will develop rapidly in the future, and valuable chicken breeds will be actively established. These will contribute to the development of poultry industries and related academic fields, and thus will eventually further human welfare.

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Ethical issues of biotechnologies in animal production (T. Heams)

Thomas HEAMS

AgroParisTech - INRAE, Paris, France.
thomas.heams@agroparistech.fr

The ethical questions raised by biotechnologies in poultry production are not limited to the important issue of animal welfare. They are at the crossroads of questions about the production activity and about what is expected from biotechnologies. To articulate this link in a relevant way, biotechnologies must be understood beyond their strict technical dimension: they are also, and perhaps above all, a revelation of our representations of the living world, blind spots included. In particular, genetic modifications on living organisms give rise to numerous ethical debates, but these are based on a sometimes-rudimentary epistemology: their moral aspect is often questioned by taking their promises for granted, whereas it is indeed the relevance of these biotechnological promises that needs to be questioned in the first place. It is only with a solid epistemology of biotechnological promises, linked to a renewed look at the dynamics of the living world, which restores their place to exchanges, plasticity, fragility and resilience, that we can establish a relevant framework for ethical discussion.

OBJECT-ORIENTED SESSIONS

Food security

Efficiently and sustainably nourishing people and poultry in the 21st Century: challenges and opportunities (R. Alders)

Robyn ALDERS

Robyn Alders (1,2,3), Giacomo de' Besi (4), Rosa Costa (2), E. Fallou Guèye (5), Johanna T. Wong (6), Soshe Ahmed (7) and Clarisse Ingabire (4)
(1) Global Health Programme, Chatham House, London, UK;
(2) Kyeema Foundation, Brisbane, Australia and Maputo, Mozambique;
(3) Development Policy Centre, Australian National University, Canberra, Australia
(4) Food and Agriculture Organization of the United Nations, Rome, Italy
(5) Food and Agriculture Organization of the United Nations, Djibouti
(6) SEBI-Livestock, The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK
(7) Department of Veterinary and Animal Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh
Email address of corresponding author: robyna@kyeemafoundation.org

Abbreviated title: Sustainably nourishing people and poultry

Summary

Tackling the impact of climate change, multiple other environmental challenges, emerging diseases and malnutrition while also meeting society's immediate needs within national budgets is a major global challenge. Additionally, the globalization of food systems along with 19th century approaches to food pricing and siloing of sectors are placing huge stresses on farmers, especially family farmers, worldwide. Family farms represent over 90 percent of all farms globally and produce 80 percent of the world's food in value terms. Currently incomes for men and women smallholder farmers are not increasing in line with Sustainable Development Goal 2 targets despite them being key drivers of sustainable development, including ending hunger and malnutrition. To nourish a growing human population efficiently, optimally and sustainably, animal source food is an integral part of human diets (especially vulnerable groups) as it provides essential amino acids and multiple bioavailable micronutrients at concentrations that significantly reduce the quantity of plant-based foods required to meet daily nutrient requirements.

The poultry industry, together with governments, international organizations and civil society, are wrestling with complex challenges to ensure that poultry contributes effectively and sustainably to food and nutrition security. These challenges include delivering intersectoral public policies and institutional environments that will: reduce competition between people and poultry for food fit for human consumption; efficiently meet nutrient requirements according to demographic and health status of consumers while also promoting nutrient recycling at prices accessible to consumers; ensure optimal food and production safety standards and reduced antimicrobial resistance;

promote innovative approaches to achieving genetic diversity and climate-smart production while also achieving efficient resource utilization; work across value chains to ensure that poultry producers and other value chain actors have fair working conditions and remuneration; and, achieve economically, environmentally and socially sustainable poultry production. The paper focuses on family poultry production and producer resilience.

Keywords: Food and nutrition security; family farmers; food safety; sustainable diets

Introduction

The 2022 World's Poultry Congress is being held at a pivotal time in the history of agriculture and society. Globally individuals, households, communities, civil society, academia, research and development organizations together with public and private sector actors are grappling with the need to manage the impact of climate change, geopolitical crises, emerging diseases such as Coronavirus disease (COVID-19), bio- and agro-diversity loss and extinctions, freshwater overconsumption, growing numbers of malnourished individuals, soil depletion and degradation, land system change and pollution (both biological and chemical) while also meeting society's immediate needs within national budgets (Alders et al. 2018a; Fanzo et al. 2022; Rampa et al. 2019). In addition to environmental pressures, the globalization of agri-food systems along with 19th century approaches to food pricing and siloing of the sectors are placing huge stresses on farmers, especially family farmers, worldwide (Alders et al. 2018a). Family farms represent over 90 per cent of all farms globally and produce 80 percent of the world's food in value terms (Lowder et al. 2021). Currently, incomes for men and women small-scale farmers are not increasing in line with Sustainable Development Goal (SDG) 2 targets (Rampa et al. 2019). Men and women smallholder farmers are considered the key drivers of sustainable development, including ending hunger and all forms of malnutrition, however, their incomes are not increasing in line with Sustainable Development Goal (SDG) 2 targets (FAO and IFAD 2019; Rampa et al. 2019).

Despite significant growth in agricultural production globally, chronic malnutrition is increasing in most countries, irrespective of their economic standing. In terms of malnutrition, the absolute number of people facing hunger is now rising, with between 720 and 811 million affected in 2020 and as many as 161 million more than in 2019 (FAO et al. 2021). Economic downturns in 2020, including those resulting from COVID-19 containment measures, contributed to one of the largest increases in world hunger in decades, affecting almost all low- and middle-income countries (LMIC). At the same time, countries are burdened by rising numbers of individuals who are overweight or obese, with many of these same individuals suffering micronutrient deficiencies (Via 2012).

To nourish a growing human population efficiently, optimally and sustainably, animal source foods (ASF) will ideally be an integral, albeit small, part of human diets (especially for children and women of reproductive age). ASF possess a specific nutrient composition that matches human nutrient requirements, particularly during the critical first 1,000 days of life covering the period from conception through pregnancy up to 2 years of age (Dror & Allen 2011). However, in the State of the World's Children Report, UNICEF (2019) stated that poor diets are driving malnutrition in early childhood and that 59% of children aged 6 to 23 months are not fed eggs, dairy, fish or meat. Although it is not yet possible to fully account for the impact of the COVID-19 pandemic, due to data limitations, it is estimated that in 2020, 22.0 percent (149.2 million) of children under 5 years of age were affected by stunting, 6.7 percent (45.4 million) were suffering from wasting and 5.7 percent (38.9 million) were overweight. ASF could have an important role in reducing stunting and some key micronutrient deficiencies as eggs, milk, meat, and offal are all nutrient-dense food products that contain high-quality proteins and several highly bioavailable vitamins (e.g., vitamin B12, only available in foods of animal origin, preformed vitamin A, and vitamin D) and minerals (e.g., iron, zinc, and calcium) (Alonso et al. 2019).

This paper explores the complex challenges that governments, the poultry industry and civil society are wrestling with to ensure that family poultry (defined as the full variety of all small-scale poultry production systems found in the rural, urban and peri-urban areas of LMIC; FAO 2014) effectively contributes to sustainable food and nutrition security in the 21st century and that can only be successfully addressed through facilitating multi- and intersectoral policies and institutional environments.

Poultry and the Sustainable Development Goals

The increasing demand for food required to meet the nutritional needs of a growing global population and the challenges facing agriculture now and into the future are among our most important challenges in the 21st century (Wong et al. 2017). For this reason, sustainable agriculture and food were identified as the second of the UN's 17 Sustainable Development Goals (SDGs), developed in 2015 as a set of globally applicable guidelines and targets aimed at improving human life, eradicating poverty and hunger, promoting peace and prosperity, and protecting the planet (UNDP 2015). These global goals are expected to guide the actions of governments, international agencies, civil society and other institutions until 2030. The 17 SDGs integrate the three dimensions of sustainable development – economic, social and environmental – with closely interwoven targets.

The poultry sector touches directly on 10 of the 17 SDGs and the other seven indirectly. While family poultry production is not the only solution to increasing global food production (SDG 2), it can have a strong impact on the most vulnerable sectors of society and play a role in the sustainable development of communities. It is also important to note that challenges related to animal health, environmental issues, and unequal access to markets, assets and knowledge, among others, make the contribution complex, where the achievement of some goals could conflict with the accomplishment of others.

Table 1 summarises the potential direct contributions of family poultry production systems to ten of the 17 SDGs. By tackling the constraints to family poultry production efficiently, their potential contribution to sustainable development can be enhanced.

Table 1. Direct contributions of family poultry production systems to the UN Sustainable Development Goals (Alders and Pym 2009; UN 2015; Wong et al. 2017).

Direct contribution pathway of family poultry	**Sustainable Development Goal**
Increasing the availability, accessibility, utilisation and stability of supply of safe food and nutrients, and improving children's cognitive development through high-quality animal source food, especially at local and regional levels, with reduced food-feed competition	2: Zero hunger 3: Good health and well-being 4: Quality education
Family poultry are able to be kept by vulnerable groups, giving them access to a source of income. Community-supported models for family poultry, including Newcastle disease prevention activities, can provide employment, including for women, and increased production can promote rural economic growth	1: No poverty 8: Decent work and economic growth
By targeting a livestock species and production system that is largely under the control of women, improvements to family poultry production systems can preferentially benefit women, promoting their empowerment, supporting income growth and entrepreneurship helping to close inequality gaps.	5: Gender equality 10: Reduced inequalities
Efficient and sustainable use of natural resources through climate-friendly, emissions' reduction, climate change adaptation and regenerative family poultry systems while achieving adequate nutrition globally requires high-income countries to decrease food wastage and consumption of calorie-dense, nutrient-poor foods, while low-and middle-income countries need to increase their consumption of nutrient-rich foods. Family poultry food products are nutritious, locally available and generally accepted, require low inputs, and typically have a short supply chain. Good practices to improve health, welfare, breeding and feeding will improve production efficiency and ensure sustainability	12: Responsible consumption and production 13: Climate action
Extensive and semi-intensive family poultry production systems do not require land clearing, contributes positively to ecosystem health, and can reduce loss of biodiversity by being a rich pool of genetic diversity and by being an alternate protein source to bush meat	15: Life on land

Options for family poultry to respond to acute shocks

Historically, family poultry research and development focused on increasing production. However, the complex problems facing family poultry and the poultry industry involve issues beyond production and these issues have been highlighted during the COVID-19 pandemic. In many countries, this crisis exposed questions regarding how to improve the efficiency and responsiveness of value chains (including feed availability), logistics and storage, as well as questions to do with ecological sustainability, land use change, nutrition, health and social protection (Hashem et al. 2020). Going forward, it is essential that policies and strategies are based on an integrated One Health, all hazards approach that works in harmony with national and global commitments, such as the SDGs, the 2019 Universal Health Coverage Declaration and the 2015-2030 Sendai Disaster Risk Reduction Framework (Alders et al. 2020).

Reducing competition between people and poultry for food

Achieving a sustainable global food system will become more difficult as the global population of humans and our domestic animals (both companion and farm animals) increases (Alders et al. 2021; Berners-Lee et al. 2018). The current production of crops has been estimated to be enough to provide sufficient food for the projected global population of 9.7 billion in 2050 (Berners-Lee et al. 2018). However, this calculation by Berners-Lee et al. (2018) assumes very significant changes to the socio-economic conditions of many (ensuring access to the global food supply) and radical changes to the dietary choices of most (including replacing most meat and dairy with plant-based alternatives, and greater acceptance of human-edible crops currently fed to animals, especially maize, as directly-consumed human food). This analysis further found no nutritional case for feeding human-edible crops to animals, which reduces calorie and protein supplies and suggested that a 'business-as-usual' dietary trajectory would require a 119% increase in edible crops grown by 2050. If implemented, such findings will have an enormous negative impact on intensive livestock production systems, including poultry. The use of alternative feed sources, such as substituting black soldier fly larvae for soybean in poultry rations may prove feasible in more intensive family poultry production units (Heuel et al. 2021).

It is important to note that in many LMIC, raising poultry for consumption is advantageous as they are small agriculture animal units, and therefore more mobile, they can be raised using very few inputs, and scavenging and semi-intensive poultry convert non-human edible products into food (Alders and Pym 2009). They also contribute to mixed crop-livestock systems through the provision of manure and contribute to the control of external parasites and pests.

Equitable sustainable poultry value chains and circular bioeconomies

Imbalances in power dynamics have been documented in many poultry value chains, especially those involving small-scale and women producers. The global food system transformation in the 21st century must include ways of valuing the various components of the value chain that enable positive and sustained change (Van Zanten et al. 2019). A move to sustainable and circular bioeconomies should be guided by principles that include placing a value on elements such as producers' well-being and animal welfare in addition to the ecosystem services that underpin the health of people, poultry and the environment (De Rosa et al. 2021). Harmful pollution, contamination and residues will impact pricing structures negatively, while the successful re-use of products (such as feathers), that were previously considered waste will make positive contributions to the poultry bioeconomy.

Efficiently and sustainably meeting macro- and micro-nutrient requirements

ASF provide a complement of easily digestible macro- and micro-nutrients that are well-matched to the human body's needs (Adesogan et al. 2020). Critical nutrients derived from poultry organs, meats and eggs include protein, unsaturated fatty acids, haem iron, zinc, selenium, choline and vitamins A, B2, B3, B5, B9 and B12 (Marangoni et al. 2015; Réhault-Godbert et al. 2019). Efficiency in nutrient uptake is particularly important for stages of life such as infancy and early childhood, when children have small stomach capacity and high nutrient requirements; women of reproductive age, who have higher iron requirements due to regular loss through the menstrual cycle; and in pregnant or lactating women, the elderly, and during convalescence, when there is either limited or reduced intake capacity, increased nutrient requirements, or both (de Pee 2017; Biesalski and Tinz 2018).

Globally, nutritional needs are not being met, with evidence of malnutrition in almost every country. In LMIC, particularly in Africa and Asia, inadequate food quantity and quality is widespread and has long-term consequences for mothers and their children during the first 1,000 days of life (FAO et al. 2021). In high-income countries (HIC), overweight and obesity are common, although despite access to a wide range of foods and dietary supplements, micronutrient deficiencies are also widespread (Biesalski and Tinz 2018).

In both LMIC and HIC, consumption of ASF has been linked to nutrition outcomes. In LMIC, where low levels of ASF consumption are common, supplementation with meat, eggs and/or milk have been shown to have positive impacts on child linear growth, micronutrient status and educational outcomes (UN Nutrition 2021). In South Asia and Eastern and Southern Africa, nutrients most at risk of being inadequate in children's diets include iron, zinc, vitamin A, folate, vitamin B12 and calcium (White et al. 2021). Chicken liver and eggs are among the most suitable foods for reducing these nutrient gaps, with only 3 g of chicken liver and 35 g of eggs required to achieve 33.3% of the dietary requirement for children 6-23 months of age (White et al. 2021).

In HIC, overconsumption of ASF has been linked to increased risk of mortality, cardiovascular disease, type 2 diabetes, and cancer (Battaglia Richi et al. 2015). Conversely, individuals following vegan or vegetarian diets particularly at risk of depleting micronutrient stores (Biesalski and Tinz 2018). Globally, ASF consumption is determined by socioeconomic factors and ASF prices, which in turn are strongly associated with rates of chronic undernutrition in children, with higher ASF prices associated with higher rates of child stunting (Headey and Alderman 2019; Development Initiatives 2020).

Overall, the addition of, or restriction to, small amounts of ASFs can greatly improve the alignment of dietary profiles to nutritional needs globally. With poultry products being associated with low emission intensities and high nitrogen use efficiency in most HIC (Gerber et al. 2013; Uwizeye et al. 2020), eggs being the most affordable source of high-quality nutrients in HIC (Drewnowski 2010) and chickens being the most accessible livestock to resource-poor households in LMIC (Wong et al. 2017), poultry products are well-placed to fill nutrient inadequacies across LMIC and HIC.

Ensuring optimal food and production safety standards

Family poultry ownership has been negatively linked with child nutrition outcomes because of its potential to contribute to an unsanitary household environment (Gelli et al. 2017), leading to increased exposure of household members to chicken faeces and feather dust. In extensive systems, although the waste produced is minimal, there is high human–chicken interaction, and infants may consume chicken faeces or contaminated dirt during exploratory. Exposure to livestock via environmental contamination has been statistically associated with environmental enteric dysfunction (EED), a disorder associated with reduced intestinal absorptive capacity and undernutrition, diarrhoea, and stunting in some but not all countries studied (Alders et al. 2018b). However, in a longitudinal study conducted in central Tanzania over 2 years, no significant association was observed between keeping indigenous chickens within human dwellings overnight and linear growth performance or diarrhoeal incidences in 503 children under 5 years (de Bruyn et al. 2018).

Food safety and food quality are currently significant issues of concern for human survival. Due to various risk factors, maintaining confidence in poultry meat and eggs is critical. Despite the considerable improvement in public health sanitation and hygiene practices in HIC, the emergence of foodborne diseases related to poultry remains a persistent threat to human health, while the prevalence of foodborne illness is still severe in LMIC.

Poultry products can become contaminated at any stage during production, processing, storage, and food preparation. Bacteria-related food poisoning, excessive use of medications (particularly antibiotics) in poultry production, pesticide residues in feed, natural toxins, or environmental pollution have come into public focus. Along with the emergence and discovery of new foodborne pathogens and other food-related hazards, the need for food-safety measures has increased. Currently, LMIC face difficulties in achieving food-safety goals due to lack of infrastructure, insufficient political and administrative structures, shortage of investment in food-safety measures targeting producers and other value chain actors, and limited research activities.

Antimicrobial resistance (AMR) is a major global public health issue. Multi-resistant bacteria in animals and humans are increasingly posing a health hazard to humans and animals, impeding successful antibacterial treatment (EFSA and ECDC 2017). Considerable single discipline science has gone into intensive poultry production with bird health and welfare and other environmental considerations not being primary considerations. Widespread use of antimicrobials is routine practice on poultry farms to compensate for unhygienic environments (Belanger 2015). Bhushan et al. (2017) found that a wide range of essential and vital antibiotics for humans are used abundantly in poultry growing units. This large-scale, unregulated antibiotic use in poultry production is leading to the emergence of antibiotic-resistant bacteria in the poultry itself, which are subsequently transmitted to humans through poultry meat and eggs. Food contamination with antibiotic-resistant bacteria can be a severe threat to public health because the antibiotic resistance genes are often transferred to other bacteria, including those of human clinical significance (Adesiji et al. 2011).

Poultry manure is frequently spread on land as a fertilizer used for agricultural purposes, facilitating the horizontal transfer of antimicrobial-resistant bacteria and genes and is an efficient reservoir of antimicrobial-resistant bacteria (Hruby et al. 2018). Policies regulating antibiotic use in livestock production, including poultry, vary significantly among countries; for example, there are restrictions regulating antimicrobials in poultry in the USA and EU countries; however, compliance is not uniform (Van Boeckel et al. 2015). In November 2021, the Codex Alimentarius Commission adopted the Revised Code of Practice to minimize and contain foodborne AMR. The code establishes that the responsible and prudent use of antimicrobial agents does not include the use of antimicrobial agents that are considered medically important for growth promotion, and that medically important antimicrobial agents should be reserved for veterinary medical and phytosanitary use (FAO and WHO 2021).

Progressive alternatives to antibiotics in poultry production to optimize health, welfare, and food safety outcomes are becoming available. However, complex problems such as AMR, require a systems-based approach to poultry production processes, individual farmers, and networks. Multipronged strategies to tackle antibiotic resistance problems must be employed by the public and private sectors. Firstly, stakeholders can be educated to promote improvements in farm management, biosecurity, and vaccination strategies. Secondly, a comprehensive set of regulations and commitments can be established for the prudent and judicious use of antimicrobials, if necessary. Thirdly, stakeholders' development and promotion of stewardship programs must incorporate responsibility and accountability regarding disease prevention, control, and treatment while optimizing the prescribing, administration, and oversight of antibiotic use in poultry.

The intensive production and processing of poultry results in manure, bedding materials, hatchery wastes, on-farm mortalities, poultry by-products, processing wastewater, and bio-solids. If not well managed, intensive poultry farming can create issues of significant environmental concern. Pollutants and pathogens in poultry litter traditionally linked to environmental degradation include excess nutrients, high protozoal, bacterial and viral loads per area of land, and chemical residues in products. Industrial poultry feed is generally based on grain and its production techniques can greatly influence the critical points of environmental impact, such as greenhouse gas (GHG) emissions, global warming, pesticides, and energy consumption.

Poultry operations need to adjust their management practices to mitigate environmental pollution – ideally, production processes should be environmentally friendly, including the incorporation of circular bioeconomy principles. Small-scale family poultry production usually generates small quantities of waste. The management of this waste should focus on proper disposal (burial or composting) to safeguard biosecurity and reduce human health hazards. Well-managed extensively raised poultry have fewer impacts on global warming and biodiversity loss than commercial chicken and other livestock. Extensively raised indigenous chickens are primarily scavengers, less reliant on the supplemental feed produced elsewhere which carries a larger environmental footprint. For small village flocks, birds are mainly hardy, indigenous breeds, which are generally better able to cope with harsh natural environments than hybrid types developed through genetic selection for production traits.

Innovative approaches to achieving sustainable poultry production

Key challenges to the sustainable development of the poultry sector include:

- the emergence and spread of transboundary animal diseases, such as avian influenza, which can pose major threats to public health;
- limited access to markets (due to distance or a lack of negotiation and/or promotion skills), productive quality inputs (day-old chicks, feeds, etc.), advisory and animal health services, education, information, finance, technologies, tools, and processing facilities, which prevent family poultry producers in LMIC from benefiting from fast poultry sector growth;
- overuse of natural resources to increase short-term production, which could lower productivity and decrease biodiversity and agro-diversity in the long-term;
- higher overall GHG emissions levels due to a rise in production, even though emission intensity from the poultry sector is declining;
- greater competition with higher levels of market concentration, which will likely keep many small producers from participating in markets;
- efficiently matching poultry production with the local context including public health nutrition requirements and minimising the competition between poultry and humans for human-edible feedstuffs.

Achieving sustainable poultry production requires:

- taking an integrated and holistic approach to promote the sustainability of poultry systems, jointly taking into consideration socio-cultural, economic, and environmental dimensions;
- working by consensus and joint action, through multi-stakeholder and intersectoral dialogue where the voices of each stakeholder (especially women and men from vulnerable groups) are equally heard;
- identifying or developing and promoting the adoption of good practices tested and validated under similar resource-poor environments (including management of endemic poultry diseases, good feeding practices, sound animal welfare, promotion of responsible consumption and equitable income generation) to make the poultry sector more resilient and environmentally sustainable;
- strengthening capacity in support of sustainable practices;
- providing enabling conditions, including by developing and strengthening policies and legal frameworks fostering sustainable poultry production. This requires investment in the sector and creating incentives for good practices and family poultry producers.

Examples of innovative approaches include:

- Poultry farmer field schools (FFS): The FFS approach overcomes constraints associated with past top-down extension activities by enabling adult participatory hands-on learning and encouraging local innovation, particularly by women and youth. In poultry FFS, groups of producers meet regularly throughout the production cycle to test, validate and adapt good poultry husbandry and marketing practices fitted to local agroecosystems and socio-economic realities. FFS allow farmers to make conscious decisions and critical analysis regarding management practices in poultry and crop production, including planning, marketing, consumption and savings. Producers acquire new skills and knowledge in poultry feeding, disease prevention and entrepreneurship, among others, and establish linkages with service and input providers, researchers, extension agents and private operators to improve their enterprises, increase access to markets and generate incomes, while promoting gender equity. FFS often result in the formation or strengthening of associations and cooperatives (FAO and IFAD 2022).

- Semi-intensive rural poultry production model in Cambodia (IFAD): This innovation is based on the adoption of new sustainable practices by small-scale indigenous chicken producers, which enable them to transition their poultry production system from 'small extensive scavenging' and 'extensive scavenging' to 'semi-intensive'. The model involves a training and knowledge-sharing programme on good poultry production and health practices and the development of integrated market clusters (i.e. setting-up of poultry breeding and poultry fattening units) in village communities. The implementation of the model (i) reduced the mortality rates of indigenous chickens from 80 to 5–10 percent; (ii) improved producers' income by USD 150–200/month from poultry production (before household consumption and occasional sale); and (iii) enhanced producers' access to markets giving the opportunity to sell poultry at higher prices (FAO 2022).
- FAO Mozambique and Kyeema Foundation poultry activities to rebuild mixed farmer livelihoods in cyclone affected areas: This involved distributing dual purpose chickens developed in and imported from South Africa and setting up sustainable Newcastle disease (ND) vaccination programs. ND continues to cause significant mortalities in unvaccinated or inadequately vaccinated flocks across the world, especially in extensively raised chicken flocks. A sustainable and community-based approach to ND control in rural and resource-limited settings has identified five key elements that are now in operation in many countries in Africa and Asia:

- support and coordination by relevant government agencies and private sector suppliers for the promotion and implementation of ND vaccination programmes;
- an appropriate vaccine (preferably thermotolerant), vaccine technology (i.e. non-invasive application methods such as eye-drop or drinking water) and vaccine distribution mechanisms (i.e. reliable cold chain, and cold chain and stock monitoring);
- a range of effective extension materials and methodologies for capacity development of men and women producers, community vaccinators, extensionists and animal health personnel;
- inclusive, cost-efficient evaluation and monitoring systems covering both technical and socioeconomic indicators; and
- economic sustainability based on the commercialization of the vaccine and vaccination services and the marketing of surplus chickens and eggs.

The vaccination practice creates micro-businesses for community-based vaccinators. It improves production of ASF through improved chicken production which contributes to improved household nutrition. It has a strong element of promoting gender equality and engaging with vulnerable rural households (FAO 2014 and 2022).

Conclusions

Human civilisation is at a critical confluence as countries and industries work to reduce the far-reaching impacts of the climate crisis, biodiversity loss, growing inequity, emerging and re-emerging infectious diseases, and noncommunicable diseases. The challenge for poultry producers and allied sectors is to identify and promote production systems that enhance the health of people, poultry, and the planet. This involves enabling producers to transition away from production systems that are unable to demonstrate a net positive impact.

Poultry production makes a significant contribution to livelihoods and human food and nutrition security. In addition, bird welfare and the contributions made by family poultry to local cultures and ecosystems must be understood and valued as we work together to deliver the SDGs and a sustainable future for all.

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New insights in alternative and organic poultry production systems (C. Castellini)

Cesare CASTELLINI

Cesare Castellini (1) *, Alice Cartoni Mancinelli (1), Lucia Rocchi (1)

(1) Dept. Agricultural, Food and Environmental Science - University of Perugia – Borgo 20 Giugno, 74 06100 Perugia – ITALY
* corresponding author cesare.castellini@unipg.it

Running head: poultry organic production

Summary

Organic Rearing System (ORS) of poultry is relatively new for the poultry sector, which is mainly oriented toward intensive production. Although the presence of a strict regulation, its practical application is non-homogenous in different farms and countries, thus different feeds, genetic strains, use of outdoor runs and range enrichments are applied. In this scenario, the choice of genetic strain is particularly relevant because it affects the adaptation of animals to ORS and the main pillars of production (productive performance, animal welfare, meat quality, environmental impact). The current guidelines mainly consider the Daily Weight Gain (DWG) as indicator of adaptability. In this view, the manuscript underlines that the poultry strain, independently on DWG, contains specific factor of adaptability. Moreover, to compare strength and critical aspects of ORS and conventional systems, we suggest the use of "One welfare" approach. Within this framework, the comparisons made showed that, although ORS in some case decrease the environmental impact and have a good impact on animal and human welfare, the high land use and the lower feed efficiency of broilers are aspects that should be considered for rendering ORS more sustainable. In this scenario, the entire paradigm of ORS should be changed for enhancing all the potentiality, both in term of food production and environmental preservation, and for achieving a high standard of animal welfare, as well.

Keywords (up to 8): organic, poultry, slow-growing strains, one welfare

Introduction

In the last years, the interest in alternative and Organic Rearing System (ORS) of poultry increased. These systems are relatively new for the poultry sector, which is mainly oriented toward intensive production. ORS is ruled by strict regulations which include all organic standards, including management practices, housing requirements, minimum slaughter age, the dietary ingredients and forbidden chemical elements. However, the practical application of ORS regulation is non-homogenous in different farms and countries thus different feeds, genetic strains, use of outdoor runs and range enrichments are used. Accordingly, the definition of reliable guidelines for feed ingredients, poultry strains and health protection of animals is a crucial factor for ORS. In particular, the poultry strain is very relevant because much of the animal response (immune response, thermoregulation, kinetic activity, behaviour and welfare) depends on genotype. The current EU regulation (889/2008) permits the use of different genotypes and only suggests the use of Slow-Growing (SG) chicken strains instead of Fast Growing (FG). FG strains are represented by standard meat-type chickens that reaches the commercial weight in about 40 days of age mainly used in intensive system. The selection of FG has mainly focused on developing highly efficient animals that have progressively substituted breeds with lower performance. SG genotypes consist in heterogeneous group of animals represented by commercial chickens' strain or local strains showing lower productive performance. Nowadays, there is no identical classification of SG chickens and many European countries categorized genetic strain only measuring the daily weight gain (DWG). However, the equilibrium between the adaptation to outdoor space and the productive performance does not depend only on DWG. Birds with high body and breast weight, show welfare problems and tends to stay indoor (Dal Bosco et al. 2010) whereas some SG genotypes have too low growth rate to be economically sustainable.

The pasture availability is one main characteristic of ORS and the grass intake positively affects the quality of products by increasing the content of essential fatty acids (Gálvez et al. 2020), vitamins and antioxidants (dal Bosco et al. 2016; Cartoni Mancinelli et al. 2019). In this scenario, it is evident that the differences in the ORS regarding the genetic strain affects the main pillars of production such as the productive performance, the animal welfare, the quality and the environmental impact. So that, for defining a specific rearing system, it is important to take into account all the output involved and not only single traits. It is important to develop specific tools for the comparison of different systems (i.e. intensive vs. ORS) to give a comprehensive perception on the productive systems. In this view, "One Welfare" concept is a very interesting tool but it is not yet a working instrument for evaluating different productive options. Briefly, One Welfare concept highlights the connections between animal welfare, human wellbeing and environment issues. One Welfare helps to promote key global objectives such as supporting food security, sustainability, reducing human suffering and improving productivity within the farming sector through a better understanding of the importance of high welfare standards. One Welfare extends the approach of the One Health theme used for human and animal promoting the links of animal welfare to human welfare and environmentally friendly animal-keeping systems. The aim of this review is:

· to outline an Adaptability Index (AI) for ORS of different poultry genotypes; ·
to delineate a multicriterial approach for "One Welfare" definition.

Adaptability Index

FG genotypes are not suitable to be reared in alternative system like ORS. Unfortunately, due to the absence of strict recommendations for ORS, in some European country (i.e. Italy) there is a residual use of FG (i.e. female chickens).

The adaptability of animal to a particular environment is a very complex issue that should consider many aspects like: health, welfare, behaviour and performance. Indeed, the study of adaptability should be carrying out through a multifactorial approach, overcoming the current opinion of adaptability based solely on DWG. Castellini et al. (2016) developed an AI using 49 variables, and showed a negative correlation between DWG and chicken adaptability to the organic system. However, within the same category of animals (SG and FG) there is no correlation between DWG and AI. Accordingly, Cartoni Mancinelli et al. (2020) reported that chickens with similar DWG were characterized by different adaptability. These studies suggest that DWG is a discriminant parameter to evaluate the adaptability but the genetic strain have intrinsic factor of adaptability independent on DWG.

One welfare

The concept of One Welfare is based on the interconnections between animal welfare, human wellbeing and the environment (Fig. 1; Pinillos et al. 2016). It extends the approach of the One Health used for human and animal health. The introduction of this concept is recent, therefore there are few attempts to use it in the evaluation of different rearing systems. In particular, the links between animal welfare, human wellbeing and environment should be identified to have an assessment of how much a certain system is close to the idea of "One welfare".

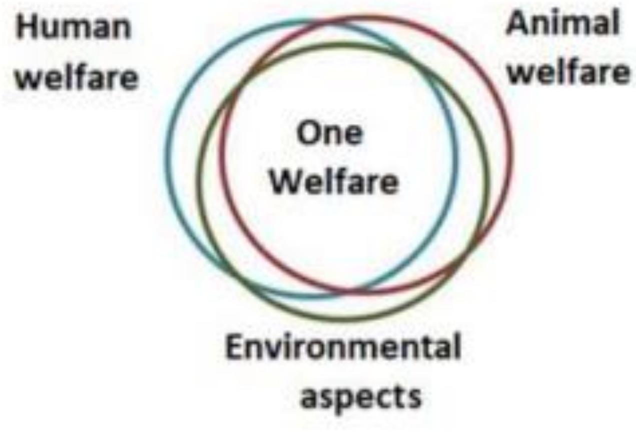

Fig. 1. One Welfare Approach (modified from Lamielle, 2010[1]).

[1] https://globalhealthvet.com/2010/08/21/about-one-health/

Multicriteria analysis

Being "One Welfare" a multidimensional concept it needs a method which can deal with these characteristics as the Multicriterial Decision Analysis (MCDA). MCDA is a family of methods, which allow to account explicitly for multiple dimensions and criteria that are also conflicting, in order to compare different alternatives (Behzadian et al., 2010; Cinelli et al., 2014). Different methods can be identified according to the theory they are based on, which determines also the possibility to avoid or not the compensation among the different aggregation of dimensions/criteria. Compensation is particularly relevant as it means to allow or not trade-off between criteria and can range from a full to a null level according to the aggregation algorithm used by the MCDA method (Cinelli et al. 2020). Moreover, MCDA allows to include the preferences and point of views of different stakeholders involved in the analysis. Till now, MCDA has been used in sustainability evaluation, which is a typical multidimensional issue, applied to several fields of research including animal rearing systems (Acosta-Alba et al., 2012; Castellini et al., 2012; Rocchi et al., 2019). Therefore, MCDA seem particular feasible to be applied in One Welfare framework context, through the construction of a proper criteria system. To this aim, we propose a MCDA-based framework based on the One Welfare approach, as a first attempt for comparing ORS with intensive system according to different indicators (e.g. environmental, social, economic, animal-based welfare criteria). The framework proposed considered three dimensions, Human and Animal Welfare and Environment, which have to be considered as the main dimensions to fully cover the concept.

Some criteria have been proposed to perform a MCDA evaluation. Table 1 shows a selection of possible criteria to be used for valuing the One Welfare. The criteria proposed have not to be considered immovable but could be modified, as long as they belong to some crucial area. Human Welfare dimension could include criteria linked to labour safety, economic revenue, meat quality and landscape. Other social criteria can be included, related social acceptability, food safety or connection with community and territory; however, they are very difficult to define. Animal welfare could take to account of some variables like: podal and sternal lesions, feathers condition, tonic immobility and animal behaviour (i.e. absence of stereotypies, active behaviours, etc.). Finally, the Environmental aspects should consider the impacts generated by the systems on the main natural matrixes (i.e.: water, air, soil, biodiversity). Additional interesting criteria are linked to the renewability of the resources employed or the effect on the soil ecosystems. As in the One Welfare framework, we cannot allow that one aspect compensate the others, thus we recommend the use of non- compensatory MCDA, which have low and null degrees of compensation. The choice of non-compensatory method is justified by the need to avoid that one option (e.g. farming system), which could be very unbalanced in some dimension, can still be successful. Electre or Promethee families, who are among of the most used MCDA methods, applied to several fields of research (Behzadian et al. 2010) could be successfully used.

Considering separately the three dimensions, according to previous studies (Castellini et al., 2012; Rocchi et al., 2019), the most critical aspects of ORS were the economic (Human welfare) and environmental criteria (e.g. land use, inorganics), whereas the ORS strengths were the social aspects and human (Human welfare) and animal welfare. If we consider separately these dimensions we cannot have a whole valuation of One Welfare whereas MCDA will give it.

In conclusion, by the way of MCDA, it is possible to compare the strength and the critical aspects of different productive systems in the “one welfare” scenario. These comparisons showed that although ORS is theoretically a solution to decrease the environmental impact of agriculture, the high land use and the lower feed efficiency of broilers are aspects that should be considered for rendering ORS more competitive and sustainable. In this scenario, the entire paradigm of ORS production should be changed as the integration of crop and livestock which has a great potential for both food production and environmental preservation, and for achieving a high standard of animal welfare.

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Diversity of production systems and services for the agroecological transition

Poultry production: A diversity of farming systems providing various services to farmers, consumers and citizens (B. Meda)

Bertrand MEDA

B. Méda (1), L. Dupuy (2), B. Dumont (3)
(1) INRAE, Université de Tours, BOA, 37380 Nouzilly, France
(2) APESA, 64053 Pau
(3) Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, 63122 Saint-Genès-Champanelle, France
Corresponding author: bertrand.meda@inrae.fr

Abbreviated Title: Services provided by poultry production
Keywords: ecosystem service, multifunctionality, systemic approach, sustainability, trade-offs

Summary

Poultry value chains are faced with the challenges of sustainable development, including a number of societal controversies (*e.g.* environmental impact, animal welfare). In this context, it is crucial to better assess the comprehensive asset base in value chains and the total range of services they provide. *Ecosystem services* can be defined as the benefits provided by an ecosystem to society. Broader definitions taking into account the multifunctionality of agriculture or livestock production have been proposed and account for the services that livestock farming systems provide to society and that are not related to ecological processes (*e.g.* employment). The concept of “bundles of services” arises when considering the various services provided by livestock farming systems that appear together repeatedly across areas and through time. Beyond the production of multiple services by livestock farming systems, there is also a need to account for their dis-services (generally called “impacts”) that are the negative externalities of livestock (*e.g.* nutrient run-off, habitat loss). In poultry production, a diversity of farming systems and species exist throughout the world, and these systems provide contrasted bundles of services. Some are oriented towards the provision of food products and territorial vitality, especially in areas with high animal density. Other bundles of services are oriented towards high-quality products that generate a positive feedback from consumers (*e.g.* free-range production). Finally, “backyard”, “family”, and “small-scale” poultry production provide many services to farmers and local communities worldwide, in particular on social aspects (*e.g.* food security, woman empowerment), despite their low productivity. Ecosystem services assessment tools are being developed, including methods for the valuation of services, to support the sustainable transition of poultry value chains in search for more “balanced” bundles of services. The “services” framework therefore represents a paradigm shift for the poultry production sector that aims to enhance its sustainability.

Introduction

Despite its important contribution to global food security (*ca.* 18 and 25% of global calories and protein consumption, respectively; Mottet *et al.*, 2017), livestock production is confronted to various controversies, notably related to its negative impacts on the environment (Steinfeld *et al.*, 2006) and animal welfare (Vanhonacker *et al.*, 2008). In the Global North, these debates went hand in hand with a decrease in meat consumption, and rise in vegetarianism/veganism (Delanoue *et al.*, 2018). Poultry nonetheless appears not to follow this trend, with a forecasted average annual growth of 2.7% in global poultry meat consumption between 2005 and 2050 (Mottet and Tempio, 2017).

Even if a strong demand for "better" systems emerged, consumers' willingness to pay more for "premium" specifications in poultry appears limited (Clark *et al.*, 2017), especially in comparison to ruminant productions. Despite the efforts made by operators to propose competitive farming systems and value chains addressing societal expectations and preserving the environment (Spoelstra *et al.*, 2013; Méda *et al.*, 2021), poultry production remains perceived negatively by a growing number of consumers and citizens for its "industrial" dimension (Delanoue *et al.*, 2018). Public institutions and non-governmental organizations (*e.g.* environment protection, animal welfare) therefore encourage citizens to become "citizen-consumers," further increasing the pressure on poultry production (Rumpala, 2011; Laestadius *et al.*, 2013).

In this context, it is crucial to better identify the total range of assets in poultry value chains, simultaneously to the range of services they provide to society. In this article, we discuss the concepts proposed to shed light on the notion of *services* provided by agro-ecosystems (AES) associated with livestock production. We explore the key concept of "bundles of services" that is closely related to coexistence, interactions and trade-offs between the various services. Services provided by different poultry production systems are then described using different conceptual frameworks. Finally, we propose ways to assess these bundles of services and help stakeholders in value chains to i) make decisions, ii) answer expectations from society, and iii) create added value at the farm and value chain levels.

What is a service? Concepts and definitions

A concept at the interface between ecology and economics

As of the seminal work from Costanza *et al.* (1997), it became clear that the world ecosystems had a value associated with the many benefits they generate for mankind. In other words, ecosystem services can be seen as the result of the attribution by beneficiaries (*i.e.* the various stakeholders using a given territory: citizens, farmers, consumers, private operators…) of a positive value to individual ecosystem functions. The Millennium Ecosystem Assessment (MEA, 2005) was constructed as a first effort to illustrate the services generated by ecosystems (Figure 1a). These services where distinguished in four categories: provisioning services (associated with the products from ecosystems: food, wood, fresh water…), regulating services (indirect services originated in the regulation functions of ecosystems; *e.g.* the role of grasslands in carbon sequestration: Soussana and Lemaire, 2014), cultural services (representing all the cultural use and non-use values of ecosystems; *e.g.* preservation of local breeds:

Beudou *et al.*, 2017) and support services (services needed to generate other services; *e.g.* nutrient cycling).

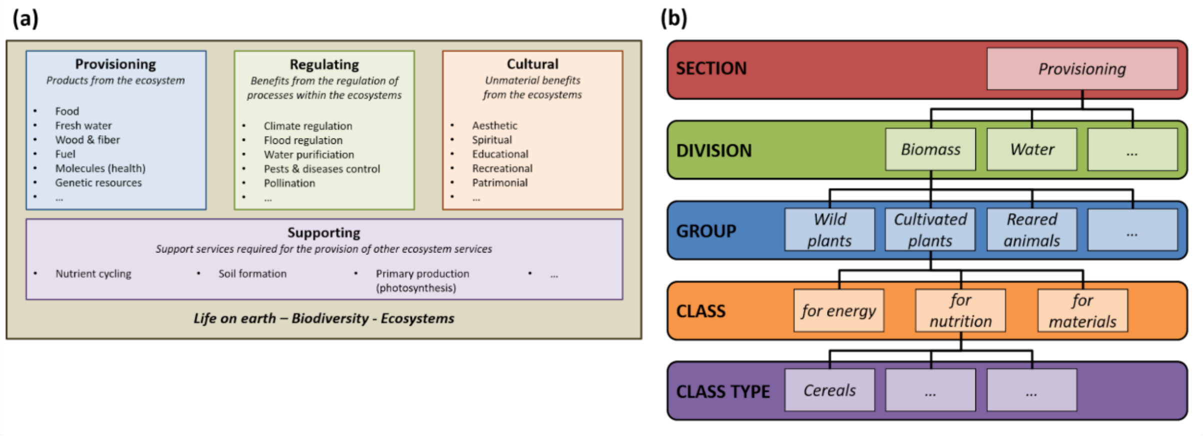


Figure 1. (a) The four categories of ecosystem services provided by ecosystems to humans proposed by the Millennium Ecosystem Assessment (adapted from MEA, 2005). (b) The structure of the Common International Classification of Ecosystem Services (taken from CICES, 2018).

However, support services have been criticized from this early stage as being different in nature and not associated with clear human needs and wants. As a result, the TEEB (The Economics of Ecosystems and Biodiversity) evaluation that followed the MEA offered to exclude support services from the list of potential services. The rationale for this was that support services are more akin to ecological functions, underlying biotic processes that govern the functioning of the ecosystem. All ecological functions should be understood as such, forming the bedrock from which a set of services can emerge. The ongoing TEEB report series, starting with the foundational work by de Groot *et al.* (2010) offered some illustrations of the values of ecosystems now understood as a stock of value (*i.e.* natural capital) from which a set of diverse services could be derived.

A final effort led by the European Environmental Agency has resulted in the creation of the Common International Classification of Ecosystem Services (CICES) which put the ecosystem services from the MAE and the emphasis on natural capital stocks and ecological functions from TEEB into a coherent classification, structured in a five-level hierarchical structure: Section (Provisioning, Regulating and Cultural services), Division, Group, Class and Class Types (Figure 1b). The CICES enables the systematic classification of ecosystem services and the clear definition of the ecological functions from which they originate. It was used as the basis for the European national inventories of ecosystem (Part of the EU 2020 biodiversity strategy) and updated to version 5.1 (CICES, 2018) to be consistent with the UN effort to extend national accounts for ecosystems (see https://seea.un.org/). The CICES is therefore the appropriate starting point to produce a branch/local level assessment of services for AES.

Impacts, dis-services, trade-offs and bundles

Acknowledging the various services provided by AES should not hinder efforts to simultaneously consider their negative effects, *i.e.* the production of “dis-services”. Dis-services correspond to nuisances for humans, resulting either from the “natural” functioning of the ecosystem (Lele *et al.*, 2013), or from negative externalities generated by human agricultural activities (Zhang *et al.*, 2007). These dis-services were not accounted for in the original MEA framework. They are also called “impacts”, as this term is generally negatively connoted.

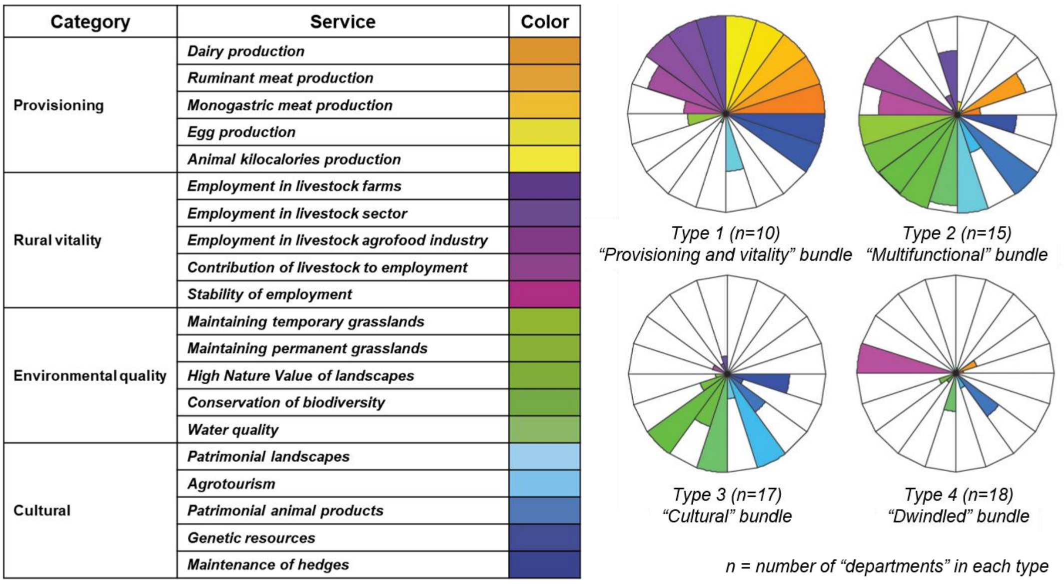
Combining the concepts of “services” and “impacts” leads to the joint consideration of the multiple effects of livestock farming. The “bundle of services” framework enables us to consider and analyze the coexistence of positive and negative effects of livestock activities that appear repeatedly across areas and through time (Raudsepp-Hearne *et al*., 2010). It reveals interrelationships between services, and interactions between underlying ecological processes (Bennett *et al.*, 2009). An “antagonism” is described when increase in one service penalizes the provision of another, or generates negative externalities. Then, a compromise (generally called “trade-off”) must be sought between the provision of services and dis-services. Conversely, when the provision of a service is favored by the provision of another service, the term “synergy” is used (Dumont *et al.*, 2019). As stressed by Tancoigne *et al.* (2014), ecosystem services have so far mainly been considered individually or in pairs, while “bundles of services” approaches remain scarce in the literature.

Towards a broader definition of the services provided by livestock

New dimensions/concepts to be integrated in approaches dedicated to livestock

Ryschawy *et al.* (2017) have first applied the concept of “bundles of services” to French livestock production. In this study, the term “services” covers both provisioning services (production of milk, meat and eggs), ecosystem services in line with the MEA (environmental quality and cultural services). However, a new category of services is considered under the name, “*rural vitality*" (*i.e.* employment, maintenance of a rural life…; Figure 2), which are essential when considering the future of livestock production (Beudou *et al.*, 2017).

Five services were considered in each of the four dimensions and quantified at the NUTS3 scale (*i.e.* French “*départements”*). At this scale, only the effects of dominant system(s) can be represented. As in other studies (*e.g.* Turner *et al.*, 2014), only the services provided by livestock farming (*i.e.* their positive effects) were represented. Nonetheless, some of the indicators can also reveal negative impacts (water pollution, odors problems) caused by livestock production when their level of provision is low. This is the case, for example, for water quality preservation, for which a low value reflects water pollution associated with livestock farming.



Category	Service	Color
Provisioning	*Dairy production*	🟧
	Ruminant meat production	🟧
	Monogastric meat production	🟨
	Egg production	🟨
	Animal kilocalories production	🟨
Rural vitality	*Employment in livestock farms*	🟪
	Employment in livestock sector	🟪
	Employment in livestock agrofood industry	🟪
	Contribution of livestock to employment	🟪
	Stability of employment	🟪
Environmental quality	*Maintaining temporary grasslands*	🟩
	Maintaining permanent grasslands	🟩
	High Nature Value of landscapes	🟩
	Conservation of biodiversity	🟩
	Water quality	🟩
Cultural	*Patrimonial landscapes*	🟦
	Agrotourism	🟦
	Patrimonial animal products	🟦
	Genetic resources	🟦
	Maintenance of hedges	🟦

Figure 2. The four type of bundles of services bundles associated to French livestock production France (adapted from Ryschawy *et al.*, 2017).

Bundles of services associated to livestock production in French territories

With this framework, Ryschawy *et al.* (2017) identified four types of French livestock production territories (groups of French "*départements*") according to the services bundles they provide (Figure 2). In particular, the "type 1" bundle corresponds to "productive" livestock farming territories (Figure 2). Thus, the "*départements*" of the Western part of France (mainly Brittany and Pays-de-la-Loire administrative regions) are all classified in this type, which highlights provisioning services (Brittany produces approximately one-third of the annual national consumption of animal proteins, excluding fish; Dourmad *et al.*, 2017) and rural vitality (numerous direct and indirect jobs created in the agricultural and agri-food sectors).

For poultry, these territories represent more than half of national eggs and poultry production, according to French statistics. However, the approach described by Ryschawy *et al.* (2017) does not distinguish conventional products from "high-quality" ones (*i.e.* associated to an official label of quality), even though "*Label Rouge*" chicken production is important in this territory (in particular in Pays-de-la-Loire region; Benoit and Méda, 2017). The "type 1" bundle described in Figure 2 also highlights a lesser expression of services contributing to environmental quality, and thus an antagonism with the provisioning services mentioned above.

The "barn", a graphical tool to synthesize the services and impacts of livestock production

Description of the conceptual framework

Consistent with the scientific literature on socio-ecological systems (*e.g.* Vallejo-Rojas *et al.*, 2016), Ryschawy *et al.* (2019) have represented livestock farming as a biotechnical system interacting with an ecological one (*i.e.* the local environment, but also climate and resources/land consumed for the production of imported concentrated feed) and a social one (labor and jobs, social and cultural issues). Livestock farming systems or value chains are represented in a pentagon, characterized by land use (permanent/temporary grasslands, crops…), farm animals (animal density and diets are schematized by their size and color, respectively), landscape elements (*e.g.* hedges) and agro-industries on which livestock production relies.

In this representation (Figure 3), livestock farming systems (or value chains) interact with five "interfaces", where pictograms symbolize elements of each "interface" that interact with livestock farming; the nature and magnitude of the effects being represented by outgoing arrows differing in their width (to represent the "intensity" of provision) and color (green = services; red = dis-services):

- "Market" interface: the incoming arrow indicates market pressures (red arrow) or opportunities (green arrow) related to specific markets (*e.g.* short circuits), while outgoing arrows represent production volumes;
- "Jobs" interface: direct jobs in livestock farms and indirect jobs (agroindustry, tourism…) are distinguished;
- "Inputs" interface: the incoming arrows indicate the use of exogenous inputs, symbolizing the delocalized effects of livestock production;
- "Environment and climate" interface: the incoming arrow indicates that livestock benefits from input services (green arrow) or suffers from dis-services or pressure from environmental factors (predation, drought…; red arrow), while outgoing arrows represent the positive or negative effects of livestock farming on the local or global (climate) environment;
- "Society" interface: the positive and negative effects of livestock production on social and cultural issues are considered.

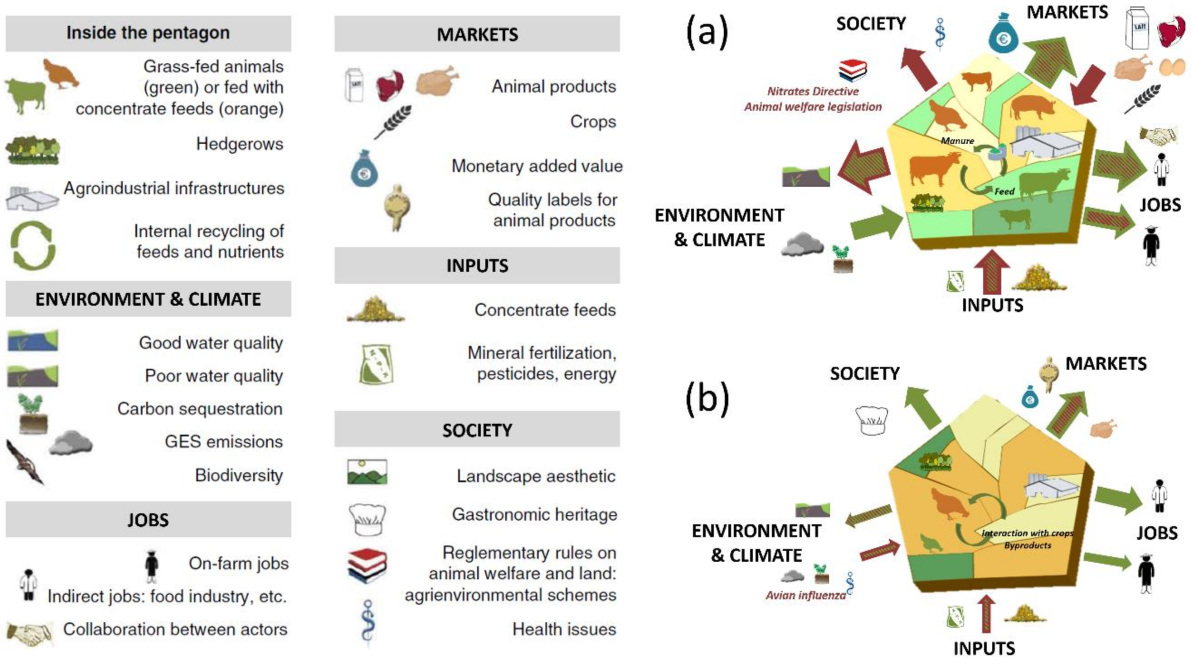


Figure 3. Services and impacts associated to (a) livestock farming in a territory with high animal density (Brittany) and (b) French free-range traditional "Label Rouge"

farming system (adapted from Benoit and Méda, 2017; Dourmad *et al.*, 2017; Ryschawy *et al.*, 2019).

In this framework, the positive and negative effects of livestock farming are explicitly represented on the same graph. This is a simplified, but educational representation allowing the comparison of livestock territories or systems, visualization of their main strengths and weaknesses, and presentation of the nature and intensity of the trade-offs to be considered. Thus, similarly to Ryschawy *et al.* (2017), Dourmad *et al.* (2017) managed to capture the trade-offs between the dis-services and provisioning services provided by the "intensive" production model in Brittany region (Figure 3a). Indeed, the high concentration of livestock farms (poultry, pigs and cattle), highly dependent on imported cereals and plant proteins (*e.g.* soybean meal), leads to increased risks in terms of nutrient leaching (Cellier *et al.*, 2014). However, the excellent animal efficiency of these productions enables the production of large volumes of affordable animal proteins with low levels of greenhouse gas and ammonia emissions per kg of animal protein (Dourmad *et al.*, 2017). Furthermore, these livestock systems/territories suffer from a poor image among citizens and consumers (Delanoue *et al.*, 2018; Sonntag *et al.*, 2019).

Finally, when applying the "barn approach" at the regional scale, the effects of niche systems still do not appear. Nevertheless, the barn allows with the same symbolism the representation of a territory (Figure 3a) or a livestock farming system (Figure 3b) or even a farm. It thus makes it possible to compare services and impacts of a dominant system with those of a niche system.

Three examples of "alternative" poultry production systems assessed with the "barn" framework

Example 1: French "Label Rouge" chicken production

The "*Label Rouge*" free-range chicken production system evaluated by Benoit and Méda (2017) using the "barn" approach of Ryschawy *et al.* (2019) in presented in Figure 3b. "*Label Rouge*" production benefits from a positive image among consumers and citizens, mostly because of animal welfare (outdoor access, lower animal density…), production conditions (slow-growing breeds, cereal-based feed…) and superior organoleptic qualities (certified by the official quality sign "*Label Rouge*").

In terms of provisioning services, "*Label Rouge*" farms represent a significant share of French chicken meat production (*ca.* 15% in 2018). Therefore, the "*Label Rouge*" sector generates a large number of direct and indirect jobs, particularly in the Pays-de-la-Loire region, which concentrates *ca.* 40-45% of national "*Label Rouge*" poultry production according to French statistics. From an environmental perspective, the lower feed efficiency of slow-growing birds results in higher greenhouse gas and ammonia emissions per kg of live weight compared to conventional systems (Prudencio da Silva *et al.*, 2014). There are also some issues regarding the risks associated with nutrient excretion on the range, in particular the accumulation of large amounts of nitrogen/phosphorus close to the chicken houses, and potential leaching to groundwater (Méda *et al.*, 2012). However, when properly managed, rangelands could also represent an opportunity in terms of connectivity between natural habitats (trees, hedges…) and maintenance of "ordinary" biodiversity (Benoit and Méda, 2017; Chiron *et al.* 2022). Furthermore, outdoor access for birds may also represent an additional health risk, in particular during avian influenza episodes (Verhagen *et al.*, 2021). Finally, regarding the use of inputs, even though "*Label Rouge*" chicken production relies on the use of concentrated feed produced outside the farms, feed is mostly made of cereals (>75%) often produced locally, hence integrating this farming system in a territorial crop-livestock loop (Moraine *et al.*, 2016). This contributes to strengthen the image of a production anchored in its territory (Benoit and Méda, 2017).

Example 2: Agroforestry association of fatty liver and walnut production in Périgord region

Based on the paper of Bijja *et al.* (2017), and using the "barn" framework of Ryschawy *et al.* (2017), we propose in Table 1 the evaluation of a French traditional agroforestry production system. In the *Périgord* region (south-west of France), geese are reared and fattened to produced fatty liver ("foie gras"). During the rearing period (until 120 days of age), geese have access to an outdoor run planted with walnut trees.

Table 1. Identification of the positive and negative aspects of agroforestry association of geese fatty liver and walnut production system described by Bijja *et al.* (2017), using the five "interfaces" of Ryschawy *et al.* (2019).

Interfaces	**Positive aspects**	**Negative aspects**
Markets	- High quality products acknowledged by official quality labels - Production of various products (fatty liver, walnuts) and by-products (wood, walnut stain…)	- Small volumes produced - Price of products (cf. "premium" labels)
Jobs	- Creation of many jobs on farms and in the local value chain (agro-food industry, tourism…)	- "Sensitivity" of the value chain during avian influenza episodes
Inputs	- Organic fertilization of trees by geese	- Use of imported concentrated feed (corn)
Environment and climate	- Contribution to weed and pest control for walnut production - Carbon sequestration (trees)	- Risks of predation and diseases (*e.g.* avian influenza)
Society	- Quality of landscapes (trees, hedges…) - Good local acceptance of production (small-scale, free-range, quality) - Contribution to local patrimony (gastronomy)	- Nuisances (odors, noise) for neighbors - Animal welfare issues (force-feeding)

Even though the primary objective of this system is the provision of food, the productivity of this system is actually low (Table 1). Indeed, in 2015, the annual production of goose "foie gras" in *Périgord* was about 350 tons (compared to about 19,000 for duck "foie gras"), even though this tonnage represented *ca.* 75% of total French goose "foie gras" production (Bijja *et al.*, 2017). Thus, in *Périgord*, this system contributes greatly to the vitality of the territory with local jobs generated both in the farms and in the local agro-industries (approximately 2,000 according to the authors) as well as a great local dynamism thanks to agro-tourism. Similarly, this system contributes largely to the cultural and patrimonial identity of the Périgord with its landscapes and its rich gastronomic heritage with food products associated to official quality labels (DeSoucey, 2010). However, the force-feeding of geese raises the sensitive question of social acceptance (Guéméné and Guy, 2004), while sanitary risks constitute a major challenge for the competitiveness of the sectors, as illustrated by the frequent avian influenza episodes (Verhagen *et al.*, 2021).

Example 3: Small-scale, family and backyard poultry production

There is a global consensus in the literature regarding the importance of "small-scale", "family", and "backyard" poultry production throughout the world (Mack *et al.*, 2005; Mottet and Tempio, 2017; Wong *et al.*, 2017). These systems may indeed represent an important share of poultry meat or egg production in some regions, especially in low-income ones (*e.g.* about 40% of egg production in Sub-Saharan Africa; Mottet and Tempio, 2017). Therefore, using the "barn" framework of Ryschawy *et al.* (2019), we propose in Table 2 an assessment of positive and negative aspects associated with these production systems.

Table 2. Identification of the positive and negative aspects of "small-scale", "family", and "backyard" poultry production using the five "interfaces" of Ryschawy *et al.* (2019).

Interfaces	Positive aspects	Negative aspects
Markets	- Production of local and fresh products - Income source for families (poverty alleviation)	- Small volumes produced in each farm (low productivity)
Jobs	- Employment of women (empowerment)	- Low contribution to local employment outside the farms
Inputs	- Recycling of many organic substrates (*e.g.* household waste) - Outdoor runs as a feed source for poultry - Production of organic manure	- Potential use of imported concentrated feed (+ high cost)
Environment and climate	- Contribution to weed and pest control in crops/orchards - Low carbon footprint	- Risks of predation and diseases (*e.g.* avian influenza)
Society	- Contribution to food security (macro/micro-nutrients), especially in developing countries - Contribution to the preservation of local breeds	- Nuisances (odors, noise) for local residents - Risks of disease transmission to local residents (*e.g.* avian influenza)

From a market perspective, these systems produce local and fresh animal products either consumed by farmers and their family or sold to local customers. When sold, these products may represent a significant increase in household income (Mack *et al.*, 2005; Mottet and Tempio, 2017). However, the productivity of these systems is quite low, and some progress margins exist (*e.g.* early nutrition, genetics, animal management; Wong *et al.*, 2017). Concerning the "Jobs" interface, poultry production in these systems is frequently managed by women, contributing therefore to their empowerment (Mack *et al.*, 2005). From an environmental point of view, these poultry production systems are generally characterized by low environmental impacts, despite a poor animal productivity. This may be explained by the fact that, in these systems, poultry is generally fed with organic wastes such as household wastes and/or with the nutrient sources available on the foraging area, when poultry have access to an outdoor run (Wong *et al.*, 2017). Moreover, the outdoor access provided to birds can also be a way of controlling weeds and pest in crops or orchards (Suh, 2014; Bosshardt et *al.*, 2022). Manure produced by poultry is also an important resource for farmers (Wong *et al.*, 2017). Conversely, the outdoor access could also represent a risk of increased mortality either by diseases or by predation (Wong *et al.*, 2017). Concerning societal issues, the production of poultry meat and/or eggs is also a great opportunity for local population, in particular children, who gain access to highly nutritive food products (*e.g.* proteins, micronutrients; Mottet and Tempio, 2017; Leroy *et al.*, 2022). However, poultry could also be perceived as nuisance (odors and noise; Delfosse *et al.*, 2017) and/or a risk (diseases; Wong *et al.*, 2017). Finally, in these systems, local poultry breeds are frequently used, therefore contributing to the preservation of domestic biodiversity (Das and Samanta, 2021).

The "services" approach: A progress tool for the poultry sector?

Thinking "services" to act "sustainable"?

The "services" approach described above is still recent. The question remains whether this conceptual framework can contribute to the sustainable development of poultry value chains. Some authors suggest that taking into account the services provided by agroecosystems could help stakeholders achieving sustainability objectives in the long term (Saidi and Spray, 2018; Wood *et al.*, 2018). This would particularly be the case when an agroecosystem provides a "balanced" services bundle. In particular, Wood *et al.* (2018) surveyed different experts on ecosystem services (academic research, development actors), and showed strong relationships between the MEA (2005) ecosystem service categories and the United Nations Sustainable Development Goals (UNO, 2015).

Moreover, a single service can contribute to the achievement of several objectives, in different sustainability "pillars". For example, in the case of free-range poultry, the presence of trees and hedges on outdoor runs can allow the production of biomass (*i.e.* provisioning service). This service can then i) contribute to the farmer's income when wood is sold (*i.e.* "economic pillar"), ii) participate to climate change mitigation via carbon storage and the production of renewable energy (*i.e.* “environmental pillar”), and iii) contribute to the beauty of natural landscapes (*i.e.* “social pillar”).

The need for practical “farm” tools: Example of the “BOUQUET” method

In the perspective of sustainable development, new farming practices should be implemented in farms. To do so, practical assessment tools should be available for stakeholders (especially farmers) to assess the direct consequences of practices on the provision of services by a given farm/farming system. Thus, in order to go further in the evaluation, and propose innovations or changes in practices (*cf.* “evaluation-innovation” cycles; Méda *et al.*, 2021), “field” tools at the farm scale are also necessary.

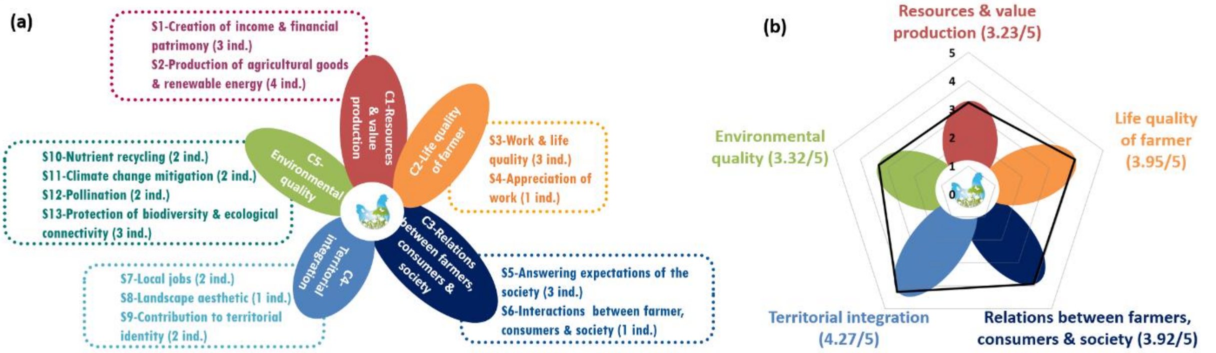


Figure 4. (a) Overview of the “BOUQUET” method for the assessment of services provided by free-range poultry systems. Five services categories (C) are declined in 13 services (S) evaluated using 29 indicators (ind.). (b) Assessment results (scores between 0 and 5) for a French organic chicken farm (adapted from Chiron *et al.*, 2022).

In this spirit, the “BOUQUET” method described by Chiron *et al.* (2022) proposes a conceptual framework for the assessment of services provided by free-range poultry systems, with the objective of helping farmers in their technical choices for the design/management of the outdoor runs. Here, the beneficiaries of the services are threefold: i) farmers, ii) consumers (of products coming from the studied farm), and iii) society. In the same vein as previous approaches, different services categories have been identified and detailed with various services. However, the categories of services are more numerous and the terms used very different from those used in the MEA framework (2005), even if concepts are similar (five services categories, declined in 13 services; Figure 4a). Unlike Bijja *et al.* (2017), the “BOUQUET” method does not consider possible dis-services generated by the farm. Following Ryschawy *et al.* (2017), the provision of dis-services can be indeed considered indirectly by a low (or insignificant) provision of a given service, in particular for the “Environmental quality” category (Figure 4a). Some concepts not covered in previous approaches were added to the investigated bundle of services. Farmers are clearly identified as the beneficiaries of certain services, such as those related to their own welfare, regrouped in the “Life quality of the farmer” category (Figure 4a). Similarly, citizens and consumers have also been considered as two different potential beneficiaries.

In order to perform quantitative assessments, the “BOUQUET” conceptual framework of Chiron *et al.* (2022) is completed by a set of 29 indicators (Figure 4a). These indicators are then weighted and aggregated at the services category level to calculate a score for each category (0 to 5; 5 being considered a “maximal” provision). As an illustration, the “BOUQUET” assessment of an organic chicken farm located in Western France (“*Deux-Sèvres*” d*épartement*) is proposed in Figure 4b. The production unit is small (480 m²) with an outdoor run of 2.7 ha planted with ash and fruit trees, central and peripheral hedges, and corn cultivated between tree rows. This farm produced a “balanced” bundle of services with a high level of provision for each category (*i.e.* scores above 3; Figure 4b), mostly due to the excellent design and management of the outdoor run (trees, hedges, crops; production of poultry, fruits and wood…). Thus, the “BOUQUET” assessment method will eventually help farmers, group technicians and agricultural advisors to better understand the central role of outdoor runs in free-range systems, and to guide decisions for a better management of those surfaces. However, this method only focuses on free-range systems. Research and development efforts are therefore still needed to propose a generic method to assess the services provided by all poultry production systems throughout the world.

Is the monetization of services a relevant tool to promote virtuous systems?

Once the issue of measuring/assessing the services provided by a farm a value chain or a territory is tackled, the question of monetizing/valuing (or not) these services arises. Monetizing a service means assigning a value to its level of provision in a given currency (euro, dollar…). This monetary value then reflects its value in the eyes of a given stakeholder (farmer, consumer, and citizen), relative to the marketable production and/or the other services generated. In free-range production, services provided by the outdoor run could be valued considering feed cost reduction (when birds consumed resources planted on the outdoor run; *e.g.* Ponte *et al.*, 2008) or the willingness of citizens to pay for diversified agricultural landscapes (presence/absence of trees, hedges; *e.g.* Notaro *et al.*, 2018). The monetization of services thus raises the question of the links between livestock production and society. Valuing services or functions currently neglected in farms implies the consideration of a broader set of societal preferences that can be directed towards agricultural production. The practical issue is then to decide how farmers can be incentivized to reorient their practices towards a “multiservice” or “multifunctional” agriculture. Monetization could also support the development of agri-environmental measures, which are still in their infancy in Europe (Baur and Schläpfer, 2018). They could become more widespread as a substitute or complement to traditional agricultural subsidies. It is also possible to imagine sanctions (*e.g.* taxes or fines) corresponding to the production of dis-services, when those are properly estimated.

The monetization of certain services is already implicitly used in studies on consumers' willingness to pay for livestock production more respectful of the environment or animal welfare (*e.g.* Clark *et al.*, 2017). There is therefore an empirical question linked to the monetization of ecosystem services related to the links between comprehensive services and the market price of animal product(s). More broadly, the distribution of the extra added value generated by these services among operators of the value chain should also be investigated. When the benefits of the services are collective, their monetization and the remuneration mechanisms allowed by this monetization will determine who will ultimately bear the cost of providing these services and benefit from its windfall.

However, this practical question should not hide the many debates that services monetization still raises. Many researchers oppose the valuation of ecosystem services for ethical and methodological reasons (Villagómez-Cortés and del-Ángel-Pérez, 2013; Temel *et al.*, 2018). One of the regularly cited risk lies in the valuation of some services (such as carbon sequestration in soil/trees) that would, because of their value, take precedence over other services that are equally fundamental to agroecosystems and to society. Objections are also regularly raised about the methods for services valuation. Based on the population's overall willingness to pay, they may ignore ecological limits or technical and scientific arguments that would make certain services "non-substitutable" compared to others, and select them as "to be preserved" regardless of their cost (Farley, 2008). Despite these persistent debates, the monetization of ecosystem services in agriculture is developing, in particular in Europe, in the context of the MAES initiative (*Mapping and Assessment of Ecosystem and their Services*) promoted by the European Commission (Maes *et al.*, 2018).

Conclusion

The concepts of "services" and "bundles of services" described in this review offers a new perspective on poultry production, taking into account the multifunctional character of agriculture. These new concepts represent a paradigm shift, which could help operators from the poultry sector to address jointly the societal (*e.g.* animal welfare), environmental (*e.g.* climate change, biodiversity), and economic (*e.g.* price and non-price competitiveness) challenges waiting ahead.

There seems to be a real interest in better assessing the services bundles provided by poultry production. Identifying and quantifying the services provided by poultry farms will enable the poultry sector to better promote them to consumers, and more broadly to the society, thus making poultry production more socially acceptable. Similarly, the assessment of dis-services/impacts and the understanding of trade-offs will help identifying relevant levers to move towards more "balanced" services bundles.

However, given the multi-criteria nature of this approach, and the need to consider multiple trade-offs, major research and development efforts are still required to propose methods and tools to i) help stakeholders in the poultry sector with their production choices (investments, farming practices…) and ii) enlighten decision-makers in the establishment of public policies for sustainable poultry farming.

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Impact of duck farming system on the environment and conservation of local genetic resources (H. Prasetyo)

Hardi PRASETYO

WPSA Indonesian Branch, Chairman of Waterfowl Working Group APF-WPSA

Email address: hardiprst@gmail.com

Abbreviated Title: Impact of duck farming system

Summary

Duck farming has been part of farming system in the villages in many parts of Asian countries, under intensive, semi-intensive, or extensive rearing system. Many different duck farming systems can be found in these areas depending on the inherited traditional practices, farming purposes, or efficiency in land uses of the local farmers. These practices resulted in various impacts on the environments and on the diversity of local genetic resources, some positive and some negative impacts, especially for the small-scale farming operations. The impacts can be seen on the temperature and pH value of the surface water, the electrical conductivity, oxidation-reduction potential, turbidity, and the contents of nitrogen (N), phosphorus (P) and potassium (K). Traditionally, majority of duck farmers in rural areas raise ducks for egg production with layer types, but with increasing demand for duck meat they suddenly switch their operations into producing duck meat by crossing their flocks with any larger available breeds without proper breeding plan. It has caused serious erosion and contamination of genetic resources within local breeds.

Key words: Duck, environment, genetic resources

Introduction

There is large variation of duck farming system in many Asian countries, either for egg or for meat production. Many different farming systems can be found in these areas depending on the inherited traditional practices, farming purposes, or efficiency in land uses of the local farmers. These practices resulted in various impacts on the environments and on the diversity of local genetic resources, some positive and some negative impacts, especially for the small-scale farming operations. It is important to be aware of these impacts in order to prevent the negative ones and benefit more from the positive ones, for the sustainability of the production and the overall farming system.

Rice–duck cultivation has become popular in Asia, it improves the ecological environment and plays an important role in reducing the impact of conventional rice farming on the environment and ecological cost of rice production. This paper discusses various effects of duck farming on the environment and genetic resources.

Effects on soil fertility.

Rice-duck farming practices are very common in many Asian countries, and they have been practiced for many generations and for many different reasons. Ducks are herded into rice fields either during early growth of rice (before flowering stage) or after harvest in order to take advantage of the available biotas as feed. Usually, this practice is conducted according to the contract agreement between the duck farmers and the rice farmers, so that both parties can take the benefit from it. Many research results and reviews have been published and only a few of them are used as references in this paper.

The rice-duck system introduces ducks into the traditional system of rice cultivation, which affects the original system on the environment. It results in the reduction of fertilizers, pesticides, herbicides and feed application due to ducks' predation, weeding and intertillage fertilization. Duck manures add nutrients to the soil, and Sawyer (2009) presented the estimated average per 1000 gallons of stored liquid pit manure a total nitrogen (N) of 22 lbs, total phosphorus (P) expressed as P2O5 of 15 lbs, and total potassium (K) expressed as K2O of 8 lbs from ducks. These values are approximation of nutrient contents of the manures, and they depend on the feed rations, bedding, and production facility. The effect of these manures of course depends very much the density of the birds and how long the birds are kept in the field. The number of studies on this aspect is still limited.

In their review on migratory birds, Fleming and Fraser (2001) stated that the fecally-derived nutrients have the potential to enrich surface water and thus contribute to the process of eutrophication, and how nutrients from migratory bird populations can affect water quality. They concluded that the impact of fecally-derived nutrient loadings in water from birds appears to vary with: bird species, bird population density, feeding habits, dilution capacity of the water body, and time of year.

From a study in Bangladesh, Hossain, et al (2005) showed that the rice-duck system is not only feasible, but also economically rewarding for the farmers. The yield of rice is, on average, 20% higher in the rice-duck system than the sole rice system. Soil analysis showed that the N, P, and K levels in the soils of the rice-duck plots were higher after cultivation than before cultivation, 10% higher N, 49% higher P, and 33% higher K. Also, the movement of ducks in the rice field enhances the aeration of the soil and prevents accumulation of harmful gases in the rhizosphere, and thus creates the stimulation of the growth of the rice plants. Result of a study in Indonesia was reported by Vipriyanti, et al (2021), and they showed that farmers using rice-duck system received higher yield and used less inorganic fertilizers, and consequently it resulted in better income. In some locations, ducks are raised above fish ponds in order to benefit from duck droppings or spilled-over feed for the fish. In practice, only catfish is suitable for this integrated farming system. The water quality of the ponds must be monitored regularly to prevent further pollution to the water system in the area.

Effects on pest control.

Being omnivorous, active and agile, ducks feed on weeds, dead leaves and pests in the field. Long, et al (2013) presented an extensive review on the subject from many published reports mostly results from China, and concluded that in general, the control effects of the system on rice planthoppers and rice leafhoppers are 60–99% and 75–100%, respectively. The effects on Chilo suppressalis and rice leafrollers are below 60%. Pesticides can be simply omitted or used once during the late period of rice growth. Further, Long et al (2013) explained that the mechanisms of how rice–duck intergrowth affects pests mainly lie in the following aspects: (i) Prevention – ducks' activities in fields improve air and light conditions, reduce relative humidity, maintain the stability of temperature and humidity, and thus help to create an environment against bacteria growth and reproduction; ducks, during their activities, smear mud on to rice stalks, protecting them against pathogenic bacteria; fertilizer application in the rice–duck cultivation system is decreased and ducks peck at ineffective tillering, so that the disease resistance of rice is enhanced. (2) Control – ducks' pecking at some sclerotia cuts down origin of bacteria; ducks' pecking destroys a few germinating hyphae which gradually shrink, stopping infection; an antimicrobial substance in duck droppings inhibits the growth and spread of bacteria. However Long, et al (2013) emphasized that the rice–duck cultivation system indeed has a notable control effect on pests in paddy fields but the results are inconsistent, and the effects are influenced by many factors such as duck age and density, fertilizers being applied, grazing period, rice type etc. Therefore, the actual system must be formulated and designed specifically for each location according to local conditions.

Teng et al (2016) also concluded from their research that in general raising ducks in the paddy fields showed a potential for controlling weed hazards, reducing rice pests and diseases, improving soil fertility, improving soil biodiversity, and increasing grain yield (1.9 times) effectively. Implementing the rice-duck integrated farming could highly reduce the amount of pesticides and herbicides and was beneficial to develop the organic rice farming in the suburbs of Shanghai, Southeast China. However, this farming practice has been restricted in some parts of Indonesia by the government, because with the outbreak of Avian Influenza ducks could serve as carriers of the virus and potential for spreading to human environment, especially in the densely populated areas. Adult birds may carry the virus without showing any obvious symptoms except for the declining egg production, and contacts with any objects during travel or in the rice fields may transmit some of the viruses. Therefore, this farming practice should be applied selectively, especially during occurrence of AI outbreak in the surrounding area.

Effects on local genetic resources.

In many Asian countries, the native ducks are generally layer types scavenging around villages, or under semi-intensive system with some area for herding. However, with the increasing demand for duck meat many farmers started to cross their flocks with larger types for meat production without any well-designed breeding programs. Some of the breeding flocks are also kept under extensive system and consequently they can mix and mate with other types or breeds. Under this situation, the genetic make-up of the native ducks might be diluted and even lost forever. Crossing among native breeds may also occur in order to obtain heterotic effects for more profitable egg production. Under a proper commercial breeding system, this production of hybrid ducks is beneficial to small farmers, but without any control or supervision farmers might deliberately cross among the hybrid ducks for replacement of their flocks. These practices are causing problems in promoting genetic improvement versus genetic conservation efforts.

The demand for duck meat is increasing in the past ten years in Indonesia, and thus the duck meat production is starting to grow rapidly every year. Traditionally, duck farmers raise native ducks for egg production only but the interest for producing duck meat is growing intensely. Consequently, farmers started to cross their flock with larger breeds, such as muscovy ducks or Pekin-like ducks, without any proper breeding plan. It has caused genetic erosion significantly among the native ducks. Also, it was discovered that crossing amongst some of the native ducks, Mojosari and Alabio ducks in Indonesia, resulted in 11.7 % heterosis in egg production (Prasetyo and Susanti, 2000), and this hybrid has been used in commercial production.

Many research results have been published on the benefit of crossbreeding between native breeds with larger breeds. In Bangladesh, crossbreeding between Pekin and Desi ducks have been used to improve body weight (Ansary, et al, 2012). In Indonesia, crossbreeding between Pekin with Mojosari ducks or with Khaki Campbell ducks were also practiced to improve growth rate for producing medium-sized carcasses which are preferred by the local market. In Korea, crossbreeding between Korean Native breed with broiler breed also produced medium-sized breed (Kim et al, 2010).

Crossbreeding between native and broiler breeds or among native breeds for better egg production have been proven to be beneficial to small farmers for improving their income and welfare. However, it should be emphasized the need for proper breeding units in the production system in order to avoid random mating among the hybrids.

Conclusion.

Majority of duck farming in many Asian countries is still under small scale operation with large variation of farming system. The existing systems prove to be profitable and adaptable to the local conditions and mostly inherited from generation to generation with some modification. However, introduced technology or methods might be required and necessary to make some adjustment in order to minimize the negative impacts of the system.

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Adapting poultry production to climate change

Combined strategies for adapting poultry production to climate change (A. Collin)

Anne COLLIN

Authors: A. Collin (1), V. Coustham (2), J.K. Tona (3), S. Tesseraud (1), S. Mignon-Grasteau (1), S. Lagarrigue (4), F. Pitel (5) and T. Zerjal (6)

(1) INRAE, Université de Tours, BOA, 37380 Nouzilly, France

(2) INRAE, Université de Pau & Pays de l'Adour, NuMeA, E2S UPPA, Aquapôle, 64310 Saint-Pée-sur-Nivelle, France.

(3) Centre d'Excellence Régional sur les Sciences Aviaires (CERSA), Université de Lomé, B.P. 1515, Lomé, Togo

(4) PEGASE, INRAE, INSTITUT AGRO, 35590 Saint-Gilles, France

(5) INRAE, INPT, ENVT, Université de Toulouse, GenPhySE, 31326 Castanet-Tolosan, France

(6) INRAE, AgroParisTech, Université Paris-Saclay, GABI, 78350 Jouy-en-Josas, France

Corresponding author: Anne.Collin@inrae.fr

Abbreviated title: Poultry production under climate change

Summary

Poultry meat and eggs are the primary sources of animal protein for human food across the world and their production has increased rapidly in the past decades. However, the poultry production chains, as other livestock sectors, are vulnerable to climate change, particularly to the global warming and its direct and indirect consequences. To face the consequences of climate change, it is necessary to build-up adaptive strategies at the animal level, to increase resilience by improving bird genetics (inclusion of indicators for general resilience to environmental disturbances in breeding goals), physiology (thermoregulation, efficiency for meat and egg production) and health. It also requires finding solutions at the system production level to understand the shifts in the geographic ranges of diseases related to climate change and to introduce mitigation practices to reduce energy consumption and greenhouse gases emissions. These strategies have to take into account the growing social demand for ethical animal productions in the One Health and One Welfare perspectives and to limit the competition between human and animals for food under climate change.

In this context, interdisciplinary research is needed. Projects focusing on genetics, technical methods (such as early thermal programming), engineering solutions, innovative nutrition and breeding strategies are being developed. They aim to improve poultry thermoregulatory abilities, housing facilities, the design of outdoor areas, and address the issue of water availability, feed efficiency, the use of suboptimal feed resources and by-products or newly available feedstuffs (insects, etc.). Some strategies favour circular economy and species associations for improving the feeding system efficiency, limiting parasite expansion, and decreasing the nutritional dependency to soya thus restricting the adverse effects of its production on deforestation and biodiversity. The present review provides some examples of levers of improvement and adaptive strategies to make poultry production systems more resilient in the context of climate change.

Keywords: heat tolerance, nutrition, genetics, epigenetics, sustainability, circular economy, biodiversity, One Welfare

Text

Livestock are key to food security, as highlighted by FAO (2017). Meat, milk and eggs provide 34% of the protein consumed across the world. Mainly, they contribute to cover the human needs in essential micronutrients such as B vitamins (notably B12 and riboflavin) and iron, zinc, and calcium. Among animal products, poultry meat and eggs are the primary sources of animal protein for human food globally. Indeed, their production has increased rapidly in the past decades. From prospective data of OCDE and FAO, it is planned that poultry production continues to progress, in developing countries (poultry meat representing 40% of the increase in meat production), and to a lesser extent in developed countries (+15%; OECD/FAO; 2020). This increase will continue to be the primary driver of meat production growth, however to a relatively lesser extent than during the past decade. Its advantage lies in its short production and commercialisation cycles, made possible by dramatic and continuous advances in genetics, animal health, and feeding practices. Productivity gains have been made in China, Brazil, and the United States, whereas in the Eastern European Union lower production costs were made possible by recent investments.

The poultry production, as other livestock sectors, are vulnerable to climate change, particularly to the global warming and its direct and indirect consequences. Among the direct consequences of climate change can be cited a rise in average environmental temperatures and more extreme weather events (Masson-Delmotte and Zhai, 2022) including extended hot spells, dry periods, an increased risk of altered precipitations, the melting of glaciers and flood risks, ocean warming and acidification. Furthermore, the mean higher temperatures favor parasitic diseases with diffusion in new zones not yet reached, and may also increase the range of reservoir hosts and vector abundance.

A major challenge with poultry farming in tropical regions is the persistent of high ambient temperatures that cause heat stress. The increased temperature has also an adverse influence on broiler and layer behaviour, physiological, and immunological responses, resulting in undesirable outcomes such as immune suppression, endocrine problems, and electrolyte imbalance, all of which impair poultry productivity (Quinteiro-Filho et al., 2012; Lara and Rostagno, 2013). Heat stress, according to Ibtisham et al. (2017), has a detrimental impact on cereal grains and causes poor feed quality and negatively affects broiler and layer productivity having an impact on tropical poultry production and, as a result, on food security. Global warming is a complex phenomenon and often the activities that are affected by it contribute to it at the same time. Poultry production, despite the feed efficiency gained through selection, the efficacy of the production systems and the possibility to use the manure for soil enrichment, contributes to greenhouse gas production, water use and the decline in biodiversity. This occurs mainly through the feed production process, energy use and animal rejections (gas volatilization) contributing to climate change, for which solutions are also proposed.

To cope with the consequences of climate change, it is therefore necessary to build-up and whenever possible, combine adaptive strategies at the level of the animal, the farm, the territory and the production chains, and to use mitigation practices to reduce energy consumption and greenhouse gas emissions.

1- First strategy: rearing animals with higher adaptive capacities

Heat stress has become a major threat for poultry production and for food security in general as global warming progresses. Undeniably, the constant increase in the productivity of modern broilers and layers has increased the susceptibility to heat stress of modern commercial breeds (Chen et al., 2009) with deleterious impacts on production, quality and efficiency traits. At the animal level, a first strategy will aim at improving climate resistance by improving bird genetics including adaptive traits in selection strategies, and resilience through early heat acclimation strategies.

1.1- Improving poultry thermoregulatory abilities for breeding chickens and hens with better heat resistance

Research is active to look for genetic solutions to improve heat resistance. Many studies, both in broilers and in layers, examined the effects of genes involved in feather coverage and quality as naked neck (NA), scaleless (sc), and frizzle (F), in improving heat resistance by reducing feather coverage increases surface of heat dissipation. The results are unanimous showing that reducing feather coverage increases heat dissipation and by consequence improves heat resistance (Chen et al., 2004; Cahaner et al., 2008; Zerjal et al., 2013). Yet, their application is limited probably due to the difficulty of introgressing rapidly a mutation in commercial lines without affecting their genetic value, and by a reticence from consumers to use products from un-feathered or naked neck birds.

Conventional breeding methods to enhance heat tolerance had found no application in poultry breeding, mainly due to the lack of easily measurable traits correlated to heat resistance and to the potential genetic antagonism between improved resistance to heat, growth and production traits (Bohren et al., 1982). Among the parameters used for evaluating the physiological impact of thermal stress, body temperatures, recorded as rectal (RT) or surface temperature (ST), have been studied to determine if they could be used as selection criteria for heat adaptation in poultry. In an advanced intercross line of broilers (heat-sensitive) × Fayoumi (heat-resistant), exposed to daily heat cycles, very low RT heritabilities, ranging between 0.03 to 0.11, were obtained, and heritabilities not statistically different from zero were obtained for changes in RT after acute and chronic heat exposure (Van Goor et al., 2015) giving little hope on the possibility of using RT as a selective criterion. Larger heritability values on the range of 0.2, were obtained in commercial layers exposed to cycles of high ambient temperature (28.4°C) for shank ST, measured by infrared thermography, suggesting that ST could be an interesting trait for selection to improve heat resistance (Loyau et al., 2016b).

Tolerance to heat stress is a complex trait as heat stress triggers the activation of complex molecular networks, such as signal transduction, expressions of heat stress-associated genes, and metabolite production. Therefore, to improve heat stress tolerance, a good knowledge of the different physiological and genetic mechanisms involved in the heat stress response is needed. For the latter, particular attention is now devoted to understanding the regulatory roles in response to heat stress of long non-coding and small RNA genes as they are important players of the complex regulatory network of protein-coding genes (Zhu et al., 2019; Dou et al., 2021). The advances in biotechnological tools allowing to produce large scale omics data, open unique opportunities to improve heat stress tolerance in chicken. Three main types of studies using omics data can be found in the literature: 1) genome-wide association analysis (Wolc et al., 2019; Zhuang et al., 2019); 2) research of selection signatures between adapted and non-adapted breeds to harsh environments (Walugembe et al., 2019; Gheyas et al., 2021); 3) analysis of differential gene expression using different tissues and/or different breeds (Lan et al., 2016; Srikanth et al., 2019). These three types of studies revealed very often concordant gene functional analyses indicating oxidative stress, metabolic processes, biological regulation, or immune responses as the most common biological processes. Identifying the genes that react to heat to maintain homeostasis and reduce issues caused by oxidative stress combined to a better understanding of the regulatory elements of the genome, may allow to identify favourable variants that could be used as biomarkers in animal breeding strategies. In this quest for adaptive variants, a fundamental role is played by local breeds, which, although much less productive than commercial lines, retain the genetic and perhaps epigenetic memory of a long-term selective pressure from the environment in which they have lived. Thus, local breeds, so far rarely used by commercial breeders as economically unattractive, could represent a valuable genetic reservoir to improve heat resistance in poultry.

A large number of expression studies have highlighted the potential role of Heat shock proteins (HSP) as stress response biomarkers to estimate the mechanism used for maintaining cellular integrity against stress damage. Increased HSP expression levels are described in heat-resistant breeds (Cedraz et al., 2017) but their application in heat tolerance selection strategies is not straightforward considering the great diversity in responses depending on the type of stress (acute versus chronic) and on the analysed tissues and breeds (Shehata et al., 2020).

To date, the molecular information used in commercial livestock selection schemes corresponds to DNA nucleotide variations fitted in additive models but these are too simplistic to represent the complexity of the genome-to-phenome relationship, even more if we consider that the capacity of organisms to adapt to a changing environment is not exclusively under genetic control. Growing evidences are highlighting the prominent role of epigenetics in gene regulation, which confers the required phenotypic plasticity to respond rapidly to environmental factors (Tzschentke et al., 2002; Kisliouk et al., 2017). Future models used in genomic selection should represent this complexity. This is a key element when breeding companies distribute their breeding stock worldwide, in particular under different climates. The acceleration of the genetic improvement of economically and sustainable important traits is necessary to supply breeding stocks that are able to express their optimal performances under a wide range of production environments. It is therefore necessary to estimate the part of the phenotypic variation due to the environment, which is largely determined by interactions between genetic and non-genetic factors. It is by using genotypic and environmental information that we will be able to better predict phenotypes.

1.2- Improving poultry thermoregulatory abilities by thermal manipulations during egg incubation

One of the environmentally-induced phenotypic variations that could be an efficient lever for adapting poultry to climate change is the altered physiological response of birds after hatching induced by thermal manipulation during embryogenesis (TM). The thermal manipulation is an early phenotype programming strategy that consists of cyclic increases in egg incubation temperature (Loyau et al., 2015). The effects of egg incubation temperature have been extensively studied in chicken, but also in several other avian species of agronomic interest, such as turkeys, ducks and quails. Cyclic increases in incubation temperature, mimicking naturally fluctuating conditions, were found to improve thermal tolerance of male broilers while minimizing hatching defects (Piestun et al., 2008). Because of the interference between the thermoregulatory system and other body functions, TM has also been shown to alter a broader range of phenotypes. For instance, TM was shown to affect growth (Loyau et al., 2013; Vitorino Carvalho et al., 2020), muscle development (Collin et al., 2007; Piestun et al., 2009), skin vascularization (Morita et al., 2016) and immunity (Shanmugasundaram et al., 2018). Moreover, TM increased liver weight and lipogenesis gene activity in Pekin ducks (Massimino et al., 2019; Wang et al., 2014).

The treatment cyclicity and the level of temperature increase are critical parameters for TM to be beneficial to the poultry (Loyau et al., 2015). For instance, temperature increases during early embryogenesis or continuously higher incubation temperatures are usually associated with hatching defects (Piestun et al., 2008; Massimino et al., 2019; Vitorino Carvalho et al., 2020). The relative humidity in the incubator should be increased accordingly to prevent dehydration during the temperature rise (Loyau et al., 2015). Breeding age and genetics also contribute to the effectiveness of TM (Yalçin et al., 2005). Therefore, incubation parameters must be fine-tuned to achieve favourable effects, which may explain why this seemingly simple procedure is not yet widely used in poultry hatcheries. The molecular mechanisms underlying the effects of TM are not yet well understood. However, recent genome-wide studies suggest that TM can affect the response of muscle and hypothalamic genes during thermal insult (Loyau et al., 2016a; Vitorino Carvalho et al., 2021). A recent study suggests that the mechanisms are likely of epigenetic nature as TM impaired two histone post-translational modifications in the hypothalamus of male broilers, with nearly 800 differentially enriched regions for the histone mark H3K4me3 that could be identified at 35 days of age between MT chickens and controls (David et al., 2019). The epigenetic regulation of thermal control is supported by studies showing that several epigenetic marks are involved in the response to postnatal heat conditioning (3 days post-hatch) during the establishment of thermal control in the brain (Yossifoff et al., 2008, Kisliouk et al., 2010).

Epigenetic mechanisms are known to be potentially transgenerational, meaning that the effects of TM could last longer than in the treated generation (Guerrero-Bosagna et al., 2018). A multigenerational effect of heat stimulation during embryogenesis was first evidenced in fast growing chickens, with a positive effect of ancestor embryonic treatment (in F0) on growth (8% body weight increase at slaughter age) and body temperatures (lower at 5 days of age) in F2 offspring untreated for two consecutive generations (Collin et al., 2018). Unpublished data in quail further supports the transgenerational effects of TM, with a persisting decrease in body weight of quails and eggs laid from the third untreated generation (Vitorino Carvalho et al., in prep). However, while we found that TM improved survival to an acute heat challenge a few days after hatching in quails, this effect did not appear to be transgenerational (Vitorino Carvalho et al., in prep). This observation calls into question the possibility of transgenerational programming to confer heat tolerance on terminal lines, a strategy that would allow TM to be combined with other practices such as on-farm hatching of commercial broilers.

Finally, a way to fight against climate change by increasing broiler resilience would be to favour the early adaptation of birds to cold, especially at young age when energy used for heating is the most important. Hence decreasing ambient temperature at start from 4°C (32 to 28°C) has enabled a decrease in gas use by 11% in experimental facilities but an increase in electricity use by 4% for ventilation (Nyuiadzi et al., 2017). The cold embryo stimulations just before hatching had positive effects on the male chicken performance, even under cold starting environment. However, in females the results were less positive, and some welfare parameters (pododermatitis score and occurrence of white striping of the breast meat) were degraded in chickens cold-exposed during embryogenesis and at start (Nyuiadzi et al., 2020). This suggests that such strategies should be carefully fine-tuned and tested on multiple criteria including animal welfare before being proposed to the poultry production chain.

2- Engineering solutions for limiting water use and favouring poultry heat loss under hot climate while limiting energy use

One means to improve the evaporative cooling in poultry houses during hot spells, hence limiting air temperature by increasing the relative humidity, is to evaporate water into the building air. On this purpose, either evaporative cooling pads or fogging and misting systems can be used in closed poultry houses, requiring water and energy.

Water demand forecasting suggests an increase in water consumption is expected to increase by 55% by 2055 (Wada et al., 2016). This is why innovations for sparing water while allowing the broilers to lose heat are currently proposed. For instance, surface wetting for direct cooling hence favouring the broiler heat loss can be achieved by sprinkling the surface of poultry with coarse water droplets (Liang et al., 2020). By this means, evaporation occurs locally on the animals, allowing evaporative heat loss from the chicken surface (Ikegushi et al., 2001; Wolfenson et al., 2001). The thermal resistance of chickens' feathers was shown to be much decreased when wet as compared to dry (Webb and King, 1984), and Mutaf et al. (2009) demonstrated the efficiency of cooling laying hens by intermittently sprinkling water at the level of the head and appendages. Liang et al. (2020) described a 66% lower water consumption by sprinkler cooling than by a conventional evaporative cooling system on five flocks over three summers in Arkansas.

Additionally to improvements concerning water use, some studies have enabled advances on optimal ventilation for regulating air velocity depending on ambient temperatures. For instance, air velocity of 1.5 to 2.0 m/s at 35°C, 60% RH, resulted in optimal convective heat loss without affecting too much body water balance (Yahav et al., 2001b; 2004).

For limiting energy use, efficient heating systems should be used in conventional systems for broilers. Air exchanger recovering heat from exhaust air to warm up the incoming air enables to gain considerable amount of energy, which could be combined with welfare-improving systems with lower stocking density without increasing the global warming potential as suggested by Leinonen et al. (2014). Besides the conception of poultry buildings favouring insulation for temperature regulation, many technological advances can also improve the fine tuning of environmental parameters (ambient air temperature, relative humidity, air velocity…) while keeping low the ammonia content indoors, limiting energy use for heating, and maintaining the litter as dry as possible. Non-negligible improvements of microclimate surrounding the farm building can be gained by building orientation, but also by planting trees or placing wind protection structures in the surroundings, which can also provide shade for limiting air temperature for animals when reared on a free range. Regarding this last practice, new low input outdoor farming systems have been designed to combine the benefits of shade and protection of laying hens against avian predators by orchards, while the birds can help fight the tree insect pests in agro-ecological farming systems.

3. Solutions to health issues triggered by climate change

Besides finding genetic, epigenetic or technical solutions to the deleterious impacts of climate change on poultry due to heat stress, health issues have also to be tackled through adaptive strategies, in a One Health and One Welfare perspective (Peyraud et al., 2019).

Climate change may affect poultry health through the arrival of pests and diseases in areas where they were not present in last decades due to the increase in temperatures that meet their optimal development conditions, in combination with an extent of commercial exchanges. For instance, the incidence of avian influenza virus outbreaks in Europe exploded during the last two years (Adlhoch et al., 2022), and this trend could continue in the future due to perturbations in migration paths of wild birds. In turn, the inadequate disposal of poultry carcasses issued from these epidemics have major impacts on local environments, especially on water quality, in some countries (Gerber et al., 2007). One solution for these epidemics would be to practice systematically poultry vaccination for avoiding mass slaughter, which requires to enhance research efforts on this purpose and to consider vaccination into the associated global trade regulation.

Additionally, both the global climate change and the popularity of backyard farming may increase the incidence of avian mite infestation as Asian poultry production expands, as recently suggested by Sparagano and Ho (2020). These authors expect the colonization of sub-tropical nations, where the seasonal temperature is comparable to tropical regions, by the Tropical Fowl Mite, and report poultry mites as potentially being vectors of other diseases (e.g. Marek disease), and a threat for other animals or human health. As acaricide resistance has enhanced recently, for example in Japan (Murano et al, 2015), biological methods such as the use of plant extracts (as garlic essential oil, Ahmed et al., 2019) or physical methods such as light regimen and gas for diminishing the mite populations could be considered (Wang et al., 2019; Kang et al., 2020).

To reduce climate change impacts on poultry production, it is also necessary to find solutions at the production system level in order to understand the geographical distribution of diseases related to climate change. This implies to revise the territorial spread of poultry production units, currently driven by the slaughter units and market sites, and to adapt poultry stocking density to limit the vulnerability of the sector to the epidemics. Accordingly, the territorial spread of breeding sites and hatcheries should be optimized to ensure local supply of chicks while maximizing the distance between such units. Installing nets around the farm buildings could also limit the risk of extension of diseases from the wild avian fauna. Furthermore, considering locally how to improve the whole production system could enable to limit live animal transport, with benefits for both energy use for transport and animal welfare.

Finally, a better farm management of manure could prevent water and food-borne disease propagation, as described by Gerber et al. (2007). This includes storing manure in closed buildings, composting it for reducing and eliminating pathogens and fly larvae, applying adequate time and rate of manure application, and improving dead-bird management and disposal.

Adapting poultry production to climate change will also require improving the environmental impacts of poultry production, thus mitigating its effects on climate change through genetic and nutritional strategies.

4. Genetic and nutritional strategies for limiting the poultry farming system-related climate change

Feed conversion ratio increases in chickens reared chronically at high temperature (Liu et al., 2020). For example, feed conversion was found to be 0.5-0.7 higher in broilers reared at 32-33°C in comparison to those reared at 22-23°C (Beaumont et al., 1998; Olfati et al., 2018). This is due mostly to a decrease in feed intake in order to limit heat production (Gous and Morris, 2005), and, to a larger extent, in a decrease in growth. The effect is especially strong in rapid growing broilers, as a high growth rate is associated with a higher heat production (Gous and Morris, 2005). Growth rate is also reduced by 25% in broilers reared at 33°C when compared to conspecifics reared at 23°C (Olfati et al., 2018). Corticosterone secreted during heat stress, apart from its negative effect on feed intake, also decreases nutrient digestibility and has a negative effect on anatomy and functionality of the digestive tract (Olfati et al., 2018).

Despite these strong phenotypic effects, the genetic determinism of feed efficiency at thermoneutral and at high temperatures seems to be quite close. Indeed, Beaumont et al. (1998) showed that heritability of Feed Conversion Ratio (FCR) was the same (0.27-0.28) at 22°C and 32°C. Moreover, the high and positive genetic correlation between FCR at 22 and 32°C (0.74) indicate that the selection on FCR at thermoneutrality would also have a positive effect at 32°C. In laying hens, Rowland et al. (2019) showed that the genetic determinism of feed efficiency related traits was also dependent on the duration of heat exposure. If during the first weeks of heat exposure, feed intake, digestive efficiency and FCR were more heritable during than before heat exposure, heritability decreased after 4 weeks of heat exposure for feed intake and FCR. The relative influence of weight gain and feed intake on final feed efficiency is also dependent on birds' age, as shown by the evolution of genetic correlations between daily feed intake, weight gain and final feed conversion ratio estimated in growing broilers by Berger et al. (2022). Even if this study did not consider heat-stressed birds, these results suggest that strategies of selection have to be adapted to each genotype by attributing different weights to feed intake, growth rate and for layers egg production to improve feed efficiency. The availability of automatic feed stations (Howie et al., 2009;

Berger et al., 2022) now opens new possibilities for selecting adapted feeding behaviors in case of heat stress. For example, it is possible to identify animals that, with the same feed intake, eat during the coolest periods of the day, thus limiting the adverse effects of feed intake on heat production.

Apart from the capacity of bird to adapt to heat stress, global warming will lead to an evolution of the composition of poultry diets, that will include a larger set of feedstuffs. Mignon-Grasteau et al. (2010) showed that selection on digestive and feed efficiency using non-optimal feedstuffs would be more efficient than when using optimal feedstuffs, as non-optimal diets challenges the capacity of birds to adapt to these diets. As the genetic correlations between efficiency traits in the two diets is highly positive, it implies that selecting on difficult diet would also improve efficiency with optimal diets. Selection strategy can nevertheless be made more complex as, depending on the new feedstuffs introduced or on the effect of high temperatures on composition of usual feedstuffs, the physiological mechanisms affected may vary. For example, Hellemans et al. (2018) showed that wheat grains contained less sulfur amino acids as cysteine and methionine when grain filling occurred during a heat episode. In this case, animals have to adapt to a different balance between amino acids in the diet. When substituting partially traditional feedstuffs by cassava pulp or macadamia nut cake, amino acid balance is not modified compared to the control diets, but the bulkiness of diet is increased, which implies that the gastro-intestinal tract capacity can be limiting as animals have to increase their intake to compensate a lower digestibility of the diet due to a faster transit (Khempaka et al., 2009; Yahav and Jha, 2021).

Many nutritional strategies have been explored for decades for managing heat exposed-chickens. The goal is thus to optimize water balance, nutrient, mineral and vitamin provision while considering special needs induced by heat stress (see the reviews by Madkour et al., 2022; Abdel-Moneim et al., 2021; Vandana et al, 2021; Renaudeau et al., 2012). These dietary approaches include for instance the use of feed additives (natural antioxidants, minerals, electrolytes, probiotics, etc.), the change of fat and/or protein contents in the diet, temporary feed restriction, and water management that will be not presented here. In the present review, we will focus on protein and amino acid nutrition, by first addressing the implications in heat-exposed chickens and then briefly providing the interest for limiting environmental impacts and global warming potential.

With respect to dietary protein concentration, studies have considered two opposite strategies to alleviate the negative effects of high temperatures on growth: 1) the use of low protein diets to limit the heat increment produced by the metabolism of protein or amino acids, and 2) the use of high protein diets to offset the decreased protein intake due to the heat-related reduction of feed intake. For the second option, higher dietary protein concentration slightly improved performance of chickens submitted to chronic heat exposure, but the effect was low and is not sufficient to help broilers to withstand hot conditions (Temim et al., 2000a, b). For the first one, data are controversial and for example, a beneficial effect was not systematically found. Indeed, providing a low protein diet (16% CP vs 20%) did not prevent the negative effects caused by heat in terms of performance, despite the addition of lysine, methionine, threonine, arginine, and valine in the diet to meet chicken requirements in the most limiting amino acids (Alleman and Leclercq, 1997). Remembering that the provision of the different amino acids is a prerequisite for maintaining optimal rates of protein synthesis is an unquestionably major concept in protein nutrition. One explanation should be that the adequate dietary AA profile should depend on the ambient temperature. For example, the arginine to lysine ratio to support performance might increase at high temperatures probably due to the reduction of arginine uptake in the digestive tract (Balnave and Brake, 2002).

Besides the role of amino acids as substrates of protein synthesis, it is crucial to take into account the functional role of amino acids. For example, methionine and cysteine are precursors of glutathione (glycine, glutamate and cysteine are the three amino acids composing glutathione) and taurine, which are essential compounds for host defense against oxidative stress (Métayer et al., 2008). Threonine and glutamine are critical for epithelial barrier function because they are involved in the synthesis of mucins and used as source of energy for the gut epithelium, respectively (Chalvon-Demersay et al., 2021; Wu et al., 2018). These functional properties of amino acids should contribute to optimal health and growth in chickens submitted to heat exposure by supporting or restoring antioxidant system, gut health or immune function (Sarsour and Persia, 2022; Abdel-Moneim et al., 2021; Wu et al., 2018; Dai et al., 2012). Other potential role of amino acids in the adaptation of chicks and market-age broilers to heat have been suggested in a recent review, pointing to citrulline and leucine, the levels of these two amino acids being affected by heat exposure (see Chowdhury et al., 2021 for details).

Protein and amino acid nutrition can also constitute a major issue for sustainable poultry production from economic, social and particularly environmental points of view. For example, formulating diets with the constraint of too high crude protein levels results in massive incorporation of soybean meal. This raw material is controversial as a feedstuff because of the price volatility, its environmental impact (deforestation, transport) and the GMO (genetically modified organisms) issue. Decreasing dietary crude protein levels can reduce the dependency on soybean but also leads to the incorporation of larger amounts of free amino acids to maintain dietary amino acid balance. It also reduces nitrogen excretion resulting from excessive amounts of undigested nitrogen or excess provision of amino acids (i.e. beyond requirements; Belloir et al., 2017; Méda et al., 2021; Cappelaere et al., 2021). This reduction of waste also allows the improvement of litter quality (e.g. lower water consumption and excretion, lower litter humidity), and contributes to a better health status of livestock. Interestingly, there is evidence that lowering dietary crude protein also contributes to reduce the climate change impact of feed in certain conditions, for instance, when Brazilian soybean meal is used (Cappelaere et al., 2021). A promising field of advances for poultry nutrition now concerns the use of insects (Józefiak and Engberg, 2015), especially larvae, which still requires research for avoiding antinutritional components, proposing highly digestible and protein-nutritive feedstuff in the context of climate change and biodiversity conservation, and potentially increasing animal welfare. However, insect production itself is a challenge for defining optimal nutrition with by-products in combination with breeding and management strategies at low-energy cost.

Some strategies are aimed at favouring circular economy and can improve the feeding system efficiency by decreasing the nutritional dependency to soya and thus restricting the adverse effects of its production on deforestation and biodiversity. Systems already associate livestock and aquaculture in integrated farms for efiiciency improvements, especially including duck rearing in Asia (Errecalde et al., 2006). However, such production systems should be carefully investigated for their environmental outputs, in terms of nutrient and mineral wastes, and pollution and biosecurity. Additionally, integrated aquaculture systems with ducks exhibit a higher prevalence of resistance genes compared with monoculture freshwater aquaculture farms as demonstrated by Xu et al. (2020).

Taken individually, the solutions proposed to adapt poultry production to climate change may have a limited impact; however, it is possible to combine them as proposed at the local level in African farm communities.

5- Combined mitigation strategies to reduce climate change impacts on sustainable poultry production: the example of African Poultry production

In Africa, it is critical to adapt poultry production to climate change to help the region face the challenge of feeding a fast-growing human population in a sustainable manner. Thus, stressful environmental elements must be minimized in order to improve poultry farming in a hot climate. This can be accomplished by employing simple design principles through management practices. For example, as management strategies, an appropriate stocking density will be used by chicken producers to maintain a pleasant temperature in the surroundings. Increased stocking density has been proven to negatively impact hen’s performance (Abudabos et al., 2013). Besides, heat stress can be addressed through proper poultry house orientation, ventilation, natural or artificial shade, and fresh water consumption. Roofs should be properly aligned, clean, and dust-free. An intermittent light regime can also help birds eat more efficiently. According to Buyse et al. (1994), broiler chicks subjected to alternating light in the poultry house through a 1L:3D lighting program create less heat at all ages, except during the compensatory growth period around 35 days old. In a study to manage feather features by selecting genes like naked neck (Na) or frizzle (F) genes (Gwaza and Nachi, 2015; Pawar et al., 2016), it was discovered that the Na gene leads to a faster growth rate and meat yield in broiler chickens under high temperatures. Its presence has been reported in Ghana (Adomako et al., 2010; Asumah et al., 2022), Nigeria (Gwaza and Nachi, 2015) and other countries and it should be used more widely in hot areas. Furthermore, the use of local more resistant breeds such as the Fayoumi breed, or of dual-purpose breeds may be more adapted to harsh rearing conditions than the conventional commercial lines.

Genetics and epigenetics can also be used to improve heat tolerance. Indeed, SNPs (single nucleotide polymorphisms) thought to be indicators for reducing heat tolerance in poultry and epigenetics can assist researchers in figuring out how environmental variables cause heritable differences in gene expression and/or genomic functions (Kong et al., 2015; Pawar et al., 2016). In line with the early acclimation strategies during embryogenesis previously mentioned, thermal manipulations of chicks appear to be one of the most promising approaches for improving broiler chicken heat resistance. Higher feed intake and stable feed efficiency are connected with the beneficial effect of early acclimation (Yahav and McMurtry, 2001a). This opens up the possibility of linking a high growth rate to increased heat tolerance. To our knowledge, early acclimation is for the moment only applied in Africa for experimental purposes, and there is a need for extension work to raise awareness among breeders or hatcheries.

Regarding feeding strategies, research showed that restriction of feed (Dale and Fuller, 1979; Attia, 2017), fat addition instead of starch, feeding time, moist feeding and diets with low protein levels (Dei and Bumbie 2011; Farghly et al., 2019) are especially recommended to reduce the detrimental effects of heat stress and improve the bird's performance. Also, in the treatment of heat stress in chicken production, phytochemicals with or suspected of having effective antioxidant effects are becoming more popular (Abioja et al. 2012; Voemesse et al., 2018, 2019). Among the phytochemicals having antioxidant properties used in Africa can be cited extracts of *Tulbaghia violacea* (35 g/kg), *Vitis vinifera* (75 mg/kg) and *Artemisia afra* (150 mg/kg) in south Africa (Naidoo et al., 2008), *Moringa oleifera* in Togo and Nigeria (Teteh et al., 2013; Daramola, 2019), *Capsicum frutescens* in Uganda (Sebulime et al., 2021), *Manihot esculenta* in Togo (Ngueda Djeuta et al., 2020) and Cameroon (Koubala et al., 2015). Finally, the feeding period should be structured so that the peak metabolic heat output does not coincide with the highest point in external temperature. One of the underlying processes of wet feeding is to increase water intake (Farghly et al., 2019).

As a conclusion, the present review has provided some examples of levers of improvement and adaptive strategies that can be combined to make poultry production systems more resilient in the context of climate change. Such adaptation strategies have to consider the growing social demand for ethical animal productions in One Health and One Welfare perspectives. This requires always considering both local situations and evaluating the multicriteria impacts of levers aiming at adapting poultry production to climate change, considering social, economic, environmental and animal welfare perspectives.

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Keel bone damage in layers

Review of keel bone damage problems and solutions (M. Toscano)

Mike TOSCANO

Center for Proper Housing: Poultry and Rabbits (ZTHZ)

Division of Animal Welfare, VPH Institute, University of Bern,

Burgerweg 22, 3052

Zollikofen, Switzerland

Corresponding author: Michael.toscano@vetsuisse.unibe.ch

Abbreviated Title: Keel bone fractures

Summary

Keel bone damage is now established as a major animal welfare and production problem within the commercial laying hen industry. Although historical evidence exists of damaged keels, the high rates of fractures and their occurrence within laying hens observed in commercial systems in the last 20 years have taken on added urgency given the likely implications for hen welfare and production. Unfortunately, the scientific and stakeholder communities lack much of the basic information needed to address the problem with effective solutions including an accepted understanding of the root cause for fractures. Despite this lack of understanding, progress has been made in key areas of assessment, identifying potential causes, and factors that affect the likelihood of fractures occurring. For instance, whereas fractures were initially assessed by palpation and/or dissection limiting resolution and/or longitudinal observations, multiple novel methods have been developed and validated including use of radiography, ultrasound, and micro computed tomography. Parallel to the development of assessment techniques, we have also established the effects of fractures on hen behaviour and productivity. Although the causes have not been identified with certainty, its now clear that high and sustained egg production, while a likely major factor, is one of several likely correlated factors. As the causal factors become more defined, effective interventions can developed with more certainty. Taken together, these advances combined with the recent attention given the problem of keel damage, indicate the issue can be resolved with continued effort and dedicated research.

Key words: hen, fracture, bone, welfare

Introduction

The high frequency of fractures seen in the keel bone of laying hens within commercial systems represents one of the greatest welfare problems facing the industry as originally suggested by the UK`s Farm Animal Welfare Committee (FAWC, 2013, 2010) and a growing body of literature (Rufener and Makagon, 2020). Beyond the obvious welfare issue of gross skeletal deformity, concern stems from the likely associated pain and suffering indicated by behavioural changes (Casey-Trott and Widowski, 2016; Rentsch et al., 2019; Rufener et al., 2019) and responses during administration of anti-inflammatory agents and analgesics (Nasr et al., 2012). Production losses have also been theorized to be a concern (Thiruvenkadan et al., 2010) with limited data estimating a reduction in egg production (Nasr et al., 2013; Rufener et al., 2018).

Keel fractures can be defined as breaks in the bone that typically manifest as a callus near the fracture site but may also involve sharp, unnatural deviations or bending (Casey-Trott et al., 2015). Recent assessment by research groups in a variety of countries indicate that levels of keel bone damage are exceptionally high, typically in excess of 50% by the end of lay (Rufener and Makagon, 2020). The problem appears to occur in many types of housing systems (Wilkins et al., 2011), including conventional and furnished cages and organic systems and across genetic lines (Rufener and Makagon, 2020). The problem has also been observed in commercial broiler breeder flocks (Gebhardt-Henrich et al., 2017).

Unfortunately, the scientific and stakeholder communities lack much of the basic information needed to address the problem with effective solutions including an accepted understanding of the root cause for fractures. Despite this lack of understanding, progress has been made in key areas of assessment, identifying potential causes, and factors that affect the likelihood of fractures occurring. Several factors have been investigated in pursuit of identifying causative mechanisms of fractures and conditions that contribute to variations in susceptibility of individual hens, with a particular focus on nutritional and environmental factors.

Causes of keel bone fracture

The theory which has attracted the greatest attention as to the source of fracture is that the high demands of calcium required of contemporary commercial hens for egg production (approximately 320 eggs over a 365-days period) induces resorption (breakdown of the bone matrix and release of contained mineral), leaving bones weak and brittle (Fleming et al., 2004; Whitehead and Fleming, 2000). While not a cause of fractures by itself, this condition leaves the hen relatively susceptible to fractures from more direct causes such as collisions with housing objects. Although the concept of high egg production draining bone mineral and weakening the hen’s skeletal structure offers a convincing mechanism to explain the increase in keel fractures, variation in the appearance of fractures over the course of the laying cycle suggests involvement of other factors and was recently reviewed (Toscano et al., 2020). Another issue causing ambiguity when evaluating the relationships between egg production and keel damage is that the majority of studies on bone mineral loss over time in laying hens have focused on the long bones as proxies for the keel. The substitution is largely due to the unusual function and shape of the keel bone, which makes biomechanical assessments difficult.

However, long bones may not respond in a uniform (Toscano et al., 2013) or even parallel manner (Toscano et al., 2015) to the keel bone, so long bone comparisons may not be relevant. Thus, while the effect of egg production is likely a major influence on keel damage susceptibility, additional investigation is required to explore the concept and the role that other factors play in the occurrence and severity of keel fractures using methods that examine the keel directly.

Strategies for intervention

Reducing the frequency and severity of keel bone damage is unlikely to be accomplished by a single change. Instead, there are multiple approaches that producers/nations should adopt as the most appropriate for their own conditions. Harlander-Mataushcek et al. (2015) provide an overview of nine specific areas for research to aid this ongoing effort. Solutions can generally be broken down into three broad categories: nutrition, housing and management, and genetic in nature.

Nutritional strategies are the simplest for implementation and likely can be applied almost immediately and adjusted as needed. Specific options include supplementation with omega-3 fatty acids to counter bone resorption and promote growth (Tarlton et al., 2013; Toscano et al., 2015) and split feeding with calcium (Pottgüter, 2016). Adjustments to housing and management are also viable options though typically require capital investment and thus should be considered more long term. A prime example is the inclusion of ramps to aid birds transitioning between tiers which have been shown to reduce fractures and collisions (Norman et al., 2021; Stratmann et al., 2015) and be a preferred form of transition even in pullets (*submitted*). These findings have led the Swiss government to require that all new aviaries installed include ramps. As a final option and that which will likely require the most time for implementation, genetic strategies can be used to phenotype birds with fracture-resistant traits (e.g., stronger keels, less hazardous activity, etc.). A major difficulty here is that phenotyping must be done in a relevant setting (e.g., cage-free) with minimal disturbance to the animals while providing high accuracy. A recent project initiated by the Center for Proper Housing of Poultry and Rabbits in collaboration with Hendrix Genetics and EW Group will advance this effort though likely require 10-15 years before dramatic changes can be seen.

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Pluridisciplinary approaches to reach the One health objectives

Poultry and One Health (S. Lamont)

Susan LAMONT

Dept. of Animal Science, Iowa State University, Ames, Iowa, U.S.A.
Corresponding author: sjlamont@iastate.edu

Abbreviated Title: Poultry and One Health

Summary

“One Health” highlights the critical intersection of multiple disciplines needed to develop the knowledge required to optimize the health of animals, humans and their environment. Poultry, people, and place are connected in a delicate balance. Poultry serves as an important source of animal protein and other nutrients to support human health. To expand the food supply to feed a burgeoning human population, it is imperative to improve efficiency and resilience of poultry in the myriad of production environments that exist around the world. Poultry contribute strongly to human health and food security in diverse regions and at diverse scales, from low-input village settings to high-input, large-scale, highly efficient commercial systems. Challenges to maintaining health can occur in all settings. The ability to maintain biological homeostasis is important for birds to thrive under different production environments, including those with suboptimal ecosystem factors, such as pathogen exposure, temperature, humidity, and feed quality. Increases in efficiency over recent decades have reduced the environmental impact of poultry, yet there are still more opportunities to increase sustainability by lowering poultry’s environmental footprint, with consideration to quality of soil, water, and air. Disease control in poultry is important for animal health as well as food safety for human health. Inappropriate use of antibiotics in animal production can contribute to the selection of microbes with antibiotic resistance, thus lowering the effectiveness of antibiotics in maintaining health of both poultry and humans. Safe and effective antibiotic alternatives are needed to support animal health, reduce antibiotic-resistant microbes in the environment, and to maintain effective treatment options for human health. Zoonotic diseases can pass from animals to humans, and vice versa. Thus, collaboration of many and diverse disciplines, including genetics, nutrition, veterinary sciences, epidemiology, agricultural engineering, management, and physiology, is essential to support and sustain global “One Health”.

Key words: poultry, humans, environment, health, zoonotic, sustainable, ecosystems

Introduction to One Health

One Health is a collaborative and poly-disciplinary approach that acts at local and global levels with the goal of achieving optimal health for animals, people and the environment. One Health has become more important in recent years because many interactions between animals, people and the environment have changed. The human population is growing and expanding into new geographic areas. Thus, there are more opportunities for close contact of poultry, livestock, wild animals, pets and people, which provides more opportunities for diseases to pass between animals and people. The increasing human population and growing economies generate the need and desire for more animal-sourced foods in the human diet. Changes in climate and land use are occurring, including expansion of intensive farming practices and establishing animal production in new areas. This can increase the environmental footprint of animal production. Disruptions in environmental conditions and habitats can also provide new opportunities for diseases to pass to domestic animals and potentially to humans. To meet consumer preferences in some regions, poultry are raised in more extensive settings that may allow greater exposure to wild birds. The movement of people, animals, and animal products has increased because of international travel and global trade and, thus, diseases can spread quickly around the globe. Important One Health issues include zoonotic diseases, antimicrobial resistance, food safety and food security, vector borne diseases, environmental footprint, and other health challenges shared by people, animals, and the environment.

Environmental footprint of poultry

Long-term sustainability of poultry production is crucial and consists of many different facets in addition to economic, environmental and social sustainability. Broom (2018) proposes the very broad definition of unsustainability as "any effect which the general public find unacceptable makes a system unsustainable, for example: inefficient use of world resources, adverse effects on human health, negative impacts on animal welfare, harmful environmental effects, unacceptable genetic modification, not being "fair trade" or damage to rural communities." As the most efficient food-producing terrestrial animals, poultry provide humans with high-quality, economical protein and micronutrients with less impact on the environment. Their small size makes them amenable to raising in many settings. Two analyses of long-term changes in the egg-layer and broiler industries demonstrate the manner in which poultry production has reduced its environmental footprint over the past several decades.

Pelletier et al., 2014, conducted a life cycle assessment of the environmental footprint of egg production in the United States, comparing 2010 with 1960, which revealed major progress by the poultry egg industry in reducing its environmental footprint over that time period. Although egg production was 30% higher in 2010 than in 1960, the footprint was lower: 63% lower in greenhouse gas emissions, 54% in acidifying emissions, 65% in eutrophying emissions, and 13% in overall energy demand. Of these reductions in environmental footprint, 28-42% was estimated as due to improved bird performance, 30-44% to changes in feed composition, and 27-30% to background systems such as improved efficiencies in transportation, energy provision, fertilizer production, and feed production. The three major factors influencing environmental impact were identified as feed efficiency, feed composition and manure management. These factors, therefore, are targets of high interest for continued improvement in footprint reduction.

To determine the effect of genetic selection on the efficiency, growth and yield of broiler 78 chickens, Zuidhof et al. (2014) compared the performance of two meat control strains that were unselected since 1957 and 1978, with a commercial broiler strain of 2005, the Ross 308. The genetic selection over the period of nearly 50 years resulted in birds with 400% increase in growth and a 50% reduction in feed conversion ratio. Over the same time, the abdominal fat percentage decreased and the pectoralis major and minor muscles greatly increased. The combined result of these changes was a reduction by one half of the feed needed to produce chicken meat, thus contributing to a substantially more efficient industry and reduced environmental footprint per unit of human food produced.

Animals, including poultry, can directly impact the surrounding environment, including watershed quality. This may occur from run-off from pasture-raised poultry or from application of manure from large-scale operations on to agricultural land. Excess nutrients, especially phosphorous, and micro-organisms can contaminate fresh water sources and impact water used for drinking and recreation and thus must be appropriately managed in poultry production (Rothrock et al. 2019).

Zoonotic diseases

Zoonotic diseases are those shared between animals and humans. Zoonoses are estimated to cause 2.5 billion human cases of illness and 2.7 million deaths annually in the world. More than 60% of known infectious diseases in humans are estimated to be potentially acquired from animals, and 75% of new or emerging infectious diseases in humans come from animals (CDC, 2022). Vector-borne diseases are increasing due to warmer temperatures and expanded habitats for mosquitos and ticks. Poultry provide nutritious food for humans but they, like any animal, may be host to a variety of micro-organisms including food-safety pathogens such as *Campylobacter spp., Salmonella spp.* and *Clostridium perfringens*. Although proper food preparation procedures eliminate these pathogens, an estimated one million people get sick from eating contaminated poultry in the United States annually (CDC, 2022). Food-borne diseases are major health issues in both developed and developing countries, with a much larger burden in developing countries that have less access to proper food storage, hygiene and food preparation facilities, and potentially more close contact between humans and animals (Abebe et al. 2020). Information about the prevalent sources of pathogens can inform effective control strategies to reduce transmission from poultry products to humans. For example, identification of table eggs as a source of a large percentage of salmonellosis cases in Denmark was followed by European Union baseline studies on laying hens and then implementation of control programs in laying hen systems that resulted in a substantial reduction in *Salmonella spp*. in laying hens (Boqvist et al. 2018). This demonstrates the One Health holistic approach of identifying an issue, collecting relevant data, communicating effectively, and making informed policies to modify practices and thereby successfully reduce zoonoses. Avian influenza is a case example of the One Health core concept of interrelatedness of environment, animals and humans. Influenza A viruses are among the most challenging ones that threaten both animal and human health. They affect a wide variety of species including the original reservoir – wild waterfowl – and domestic poultry, humans, and many other mammalian species. They evolve and adapt to new species hosts and exhibit a high frequency of inter-species transmission (Short et al. 2015). The low-pathology avian influenza (LPAI) virus subtypes H5 and H7 can evolve to high-pathology avian influenza (HPAI) in terrestrial poultry in which the viruses cause systemic and fatal infection. The evolved HPAI can again cross species barriers and be transmitted to other avian and mammalian species including humans. The emergence of avian influenza H5N1 and H7N9 in China occurred at the time of intensification of poultry production in areas with wetland agriculture and wild waterfowl habitat, providing an opportunity for extensive exposure to the wild waterfowl reservoir of avian influenza viruses (Gilbert et al. 2017). Poultry live markets supported the spread and persistence of avian influenza, as well as human exposure. The emergence of HPAI, however, has also occurred in countries with high biosecurity standards but typically also in areas of intensive poultry production. Wild waterfowl usually are asymptomatic carriers of avian influenza virus and can therefore distribute it widely during their extensive migration. Preventing bird-bird contact, and transmission of avian influenza virus from wild waterfowl into water sources and facilities used by domestic poultry, will reduce the potential for the waterfowl vector to spread the virus to domestic poultry (Elmberg et al. 2015). Because of the complexity of control measures, including human flu vaccination, personal protective equipment, manure management, and biosecurity practices, a multi-pronged approach is needed to reduce transmission of avian influenza to poultry and to humans (Moore et al. 2021).

Antimicrobial resistance (AMR)

A major issue within One Health is the development and spread of microbes that are resistant to antibiotics. If a microbial population is exposed to antimicrobials in its host organism or environment, the susceptible portion of the microbial population is killed and the small percentage that are genetically resistant then expand. In animal agriculture, antimicrobials are used therapeutically to treat individual sick animals. For practicality and efficiency, however, antimicrobials are often administered through water or feed to whole groups of poultry for prophylaxis (to healthy animals at risk of infection) or metaphylaxis (to healthy animals in the same group as diseased animals) (Collignon and Ewen, 2019). Environmental bacteria, being the most abundant organisms, serve as sources for AMR genes that can become incorporated into pathogens of people and animals. This is a naturally occurring phenomenon. However, it is accelerated by depositing microbes with AMR genes into the environment from waste of poultry, other livestock and humans, antibiotic residues from the pharmaceutical industry and hospitals and from intensive livestock farms (Robinson et al. 2016). Antimicrobial resistant (AMR) pathogens can rapidly spread through animal and human populations and the environment, making it harder to then treat future infections in animals and people. One Health-based multidisciplinary research and action is needed to stem the increase in AMR (White and Hughes, 2019). To be successful, it must be sensitive to the role that poultry and other food animal species play in livelihoods across the globe.

Conclusions

Achieving the One Health goals of maintaining and improving the health of animals, people and their environment depends upon the collaborative work of teams that represent diverse and complementary disciplines, including nutrition, physiology, genetics, veterinary and human medicine, environmental sciences, agricultural engineering, communications, and many more. Additionally, engagement of policy-makers, law enforcement and the public are needed. Because poultry species are highly efficient in food production, they can serve an important role in global food security and also in supporting "One Health".

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Understanding the microbiome for an improved management of Health and Welfare

Nutritional control of the microbiome for improved health and welfare (R. Ducatelle)

Richard DUCATELLE

R. Ducatelle, F. Van Immerseel, and E. Goossens

Dept. of Pathology, Bacteriology and Avian Medicine, Faculty of Veterinary Medicine, Ghent University, Belgium.

Corresponding author: Richard.ducatelle@ugent.be

Abbreviated title: Nutritional control of microbiome

Summary:

Massive feed intake puts a lot of stress on the physiology of the digestive system of broilers, often leading to incomplete digestion of nutrients, which in turn leads to shifts in the ileal and caecal microbiota with ensuing mucosal inflammation and barrier leakage. In the past these issues were kept under control by the use of antimicrobial growth promoters. Since the ban on antimicrobial growth promoters these issues inevitably have taken larger proportions, pushing the industry to search for alternative solutions and pushing scientists to try and better understand the delicate interactions between feed, microbes and the host mucosa. Today, a range of different tools are available to support gut health and control the microbiome in broilers. Most of these tools are additives which are mixed in the feed in small amounts and therefore do not interfere with the traditional feed formulation. They include probiotics, prebiotics, phytobiotics, enzymes, etc. Most of these additives aim at restoring a beneficial microbiota in the lower intestinal tract. Even if there are still many open questions regarding the beneficial microbiota, it is clear that increasing richness, evenness and diversity, expanding butyrate producers and suppressing Enterobacteriaceae are key targets when trying to steer the microbiota for improved intestinal health. We recently showed such effects for certain xylanase enzymes and xylooligosaccharide type prebiotics. Rather unexpectedly, we also showed similar effects for reduced particle size wheat bran. An entirely novel category of feed additives is targeting the small intestine, supporting natural antimicrobial defense and mucosal repair mechanisms, which essentially facilitates better absorption of the nutrients. In conclusion, feed formulators now are facing the challenge to choose the right additives which best comply with their feed formula under their specific conditions.

Key words: microbiome, health, welfare,

Introduction:

Decades of continuous genetic selection for breast meat yield and daily weight gain has created a broiler with an incredible appetite. Overeating indeed is a major issue in the modern broiler. The excessive feed intake puts a lot of pressure on the digestive system. This may lead to incomplete digestion and absorption of the nutrients in the small intestine, with an appreciable digestive fraction ending up in the faeces. Amongst practitioners this phenomenon is sometimes called ‘feed passage syndrome’. The altered composition of the intestinal content which is taken up by the caeca induces a pathologic shift in the caecal microbiota, often referred to as ‘dysbiosis’. Reverse peristalsis can seed this potentially harmful microbial population into the small intestine, leading to an undesirable expansion of the microbial population of the jejunum and even the duodenum. In man, a condition called ‘small intestinal bacterial overgrowth’ presents many similarities. The entire cascade of events as depicted above, leads to upregulation of inflammation along the intestinal tract. Since the venous blood flow from the intestine goes through the portal vein, it can carry these inflammatory and other signals directly to the liver. When the signals exceed a certain threshold, the liver will respond with an acute phase reaction and the production of acute phase proteins. When this situation persists, it will ultimately lead to reduced weight gain.

Since the ban on antimicrobial growth promoters, prevention and control of this cascade of unfavorable events has mostly relied on the reduction of feed intake, usually by intermittent lighting and other management interventions. The present paper, however, discusses a selection of nutritional tools to control the microbiome, through which it is possible to intervene at different levels in the above-mentioned process, leading ultimately to the stimulation of the absorption of nutrients in the small intestine, as well as avoidance of inflammatory signal transfer to the liver.

Control of the caecal microbiome:

By the time the intestinal content is taken up by the caeca, it should, in principle, only contain the indigestible fiber fraction from the feed and some proteins secreted by the host (so-called endogenous nitrogen). The fibers in chicken feed essentially are large macromolecular polysaccharides that make up the plant cell walls, including mostly arabinoxylans, betaglucans, cellulose and mannans. These polysaccharides are degraded by the enzymes of bacteria, which mostly belong to the phyla of the Bacteroidetes and the Firmicutes. The caecal microbiome, however, is more than just Bacteroidetes and Firmicutes. It is a complex ecosystem with numerous interactions between the different members as well as interactions with the host. These interactions are still poorly understood, in spite of the numerous papers dedicated to the topic. It is still a matter of debate which bacterial genera, species and strains have a beneficial role and which are potentially harmful. Nevertheless, even if the subtle modulating roles of many different bacterial species and strains are still under investigation, some dominant patterns have been clearly identified already. One such pattern is the production of butyrate by members of the Lachnospiraceae and Ruminococcaceae families. This end product of bacterial metabolism is not only a nutrient source for the epithelial cells of the lower intestinal tract, it is also a signal molecule for the host tissues (Guilloteau et al., 2010). Through the G protein-coupled receptors GPR41, GPR43 and GPR109A, the epithelial cells can sense and respond to butyrate produced by the microbiota. One subpopulation of cells, namely the L-cells, responds by increased density and the production of a.o. Glucagon like peptide 1 and 2, which control satiety and the growth of the small intestinal villi. A variety of nutritional tools are currently available to support this important metabolic pathway. Butyrate can be directly incorporated in the feed. However, it needs to be protected in order to be delivered sufficiently down the lower part of the intestinal tract (Onrust et al., 2020). The beneficial effects of this approach have been documented as shown a.o. in the paper by Onrust et al. (2020). The endogenous production of butyrate in the caeca is derived from the microbial fermentation of fiber. Due to the heterogeneity of the macromolecular complexes that make up the plant cell wall derived fibers in different feed ingredients, fermentation of fibers in the caeca require coordinated action of different bacterial enzymes often produced by different bacterial species and strains. Especially in the starter period, when the caecal microbiome is still immature, caecal fermentation can be stimulated and fueled by providing prebiotic (i.e. oligomeric fragments of fiber) as feed supplements. This strategy was demonstrated in the paper by De Maesschalck et al. (2015) by adding xylo-oligosaccharides to broiler feed. This prebiotic supplement supported the expansion of specific butyrate producers in the caeca. The process of microbial fermentation of fiber can also be fostered by exogenous enzymes added to the feed. We showed that in feed xylanases degrade the arabinoxylans from wheat into oligomers with a degree of polymerization of 15 to 30, facilitating further degradation and thereby increasing the production by the caecal microbiota of short chain fatty acids in general and butyrate in particular (Yacoubi et al., 2016). When combining a xylanase with an arabinofuranosidase, efficient caecal release of butyrate from corn arabinoxylans can be achieved (Ravn et al., 2018). These data indicate that the breakdown of complex mixtures of plant derived dietary fibers, present in feed, requires activities of different glycoside hydrolases, which may be produced by the same or by different species or strains of bacteria. These enzymes may be secreted by the bacteria in the environment with ensuing highest concentrations close to the bacterial cell, or they may remain attached to the bacterial surface. Considering the importance of close contact between the bacterial cell and the dietary fiber, attachment to the fiber may be important in the case of insoluble fibers. The question has been raised on several occasions whether size and shape of the particles of insoluble fiber may influence fermentability by the caecal microbiota. We investigated the role of particle size in the efficiency of fiber attachment and fermentation by supplementing a broiler diet with wheat bran of different particle size. These studies showed that there is an optimal particle size of wheat bran of around 280 µm which is preferentially colonized by members of the genus Lactobacillus and of the families Lachnospiraceae and Ruminococcaceae (Vermeulen et al., 2018). Lactobacilli are known for their broad and versatile spectrum of glycoside hydrolases. Lactate, the major end product of their metabolism can cross feed to members of the Lachnospiraceae family, which can use lactate for the production of butyrate.

It is obvious that butyrate is just one of a broad array of caecal microbial metabolites that play a role in the physiology of the avian caeca. Many other microbial metabolic pathways, which are involved in the breakdown of substrates available in the caecal content, have been reported to generate potentially bioactive compounds, although their function often is not completely clarified. One such pathway that is worth mentioning is the caecal microbial degradation of cellulose. By supplementing pure amorphous cellulose to the feed of broilers, we were able to show that cellulose in feed is used by microorganisms belonging to the genus Alistipes, which belong to the family Rikenellaceae of the phylum Bacteroidetes (De Maesschalck et al., 2019). A major end product of their metabolism is succinate. The succinate which is taken up by the caecal epithelium through the GPR91 receptor is not used for energy supply of the host epithelial cell, but is converted by the epithelial cell into glucose and can thus contribute to intestinal gluconeogenesis. In this way, cellulose supplementation may be an elegant means of protecting broilers from hypoglycemia. Recently, this unique role of Alistipes in the degradation of dietary cellulose has also been reported in man.

Control of the ileal microbiome:

The ileum in chickens is characterized by a moderately abundant microbiome (up to 10^9 cfu / ml) which is dominated by the genus Lactobacillus. As mentioned above, members of this genus can be involved in fiber fermentation, generating lactate as their main end metabolite. Lactate can be taken up by the epithelial cells through the GPR81 receptor, but part of it will enter the caeca, serving as a substrate for members of the family Lachnospiraceae to produce butyrate. By virtue of their versatility, Lactobacilli, however, can switch to other substrates when these are more readily available and more easily fermentable. This is the case when excessive amounts of the digestible fraction of the feed enters the ileum. It may lead to an undesirable expansion of the population upstream the small intestine. It also may lead to excessive, massive production of lactate that cannot be consumed fast enough by the caecal microbiota, possibly leading to a pH drop. A pH below 5.5 is detrimental to the caecal butyrate producers. Avialability of large amounts of the digestible fraction of the feed also may lead to an expansion of the family Enterobacteriaceae in the ileum. Control of the ileal microbiome thus should rely on improving the absorption of the nutrients at the level of the duodenum and jejunum. Stimulation of villus growth (increasing absorptive surface) and reduction of oxidative stress thus may indirectly control the ileal microbiota. This was recently illustrated in a study comparing the effects of different sources of zinc supplement in the diet of broilers (Degrande et al., 2020).

Control of the duodenal and jejunal microbiome:

This part of the intestine (especially the duodenum) is characterized by a very low abundant microbiome (< 10^3 147 cfu / ml). In man it has been shown that the microbiome of the jejunum clusters closely with the microbiome of saliva, suggesting that the maintenance of this microbiome is dependent on a continuous supply from the oral cavity. The host mucosa at the level of the duodenum and jejunum is equipped with an arsenal of powerful antibacterial defense molecules, which all contribute to the maintenance of a microbe-poor environment, especially close to the epithelial surface. These tools include the secretion by the epithelium of alkaline phosphatase, an enzyme to is capable of inactivating the lipopolysaccharide of gram-negative bacteria. The pore-forming peptidoglycan receptor protein and other antibacterial proteins and peptides secreted by the epithelium have bactericidal activity. Lysozyme secreted by Paneth cells further breaks down the bacterial peptidoglycan into its smallest bioactive fragment, muramyl dipeptide (MDP). This MDP can be sensed by the epithelium through the NOD2 receptor (NOD1 in the chicken), which, when continuously stimulated, dampens down inflammation. Efficient absorption of the nutrients by membrane bound digestion and receptor-mediated absorption, and a continuous flow of the intestinal content, propagated by powerful peristaltic waves, should further prevent excessive bacterial growth in this segment of the intestine. All of this is designed to avoid competition between the host and his microbiota for the nutrients. Nutritional tools to support the physiological processes that control the microbiota in the jejunum and ileum are currently under investigation.

Conclusion:

Today, a number of nutritional tools are available which allow steering of the caecal microbiota towards the expansion of phyla, families and genera that produce metabolites with proven beneficial effects, such as butyrate. Application of these tools will be most successful under conditions when the caecal microbiota is under pressure. It is questionable whether combining several of these tools with similar modes of action will have further additive effects. The microbial metabolic network in the caeca is, however, extremely complex and involves much more than the mere production of short chain fatty acids. It is clear that many intermediate and end products of microbial metabolism can have profound effects on the host. Unraveling these effects is the current challenge for intestinal health research. It may lead to the further discovery of novel nutritional tools to support gastrointestinal function, for the benefit of the birds' health and welfare.

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Current status of exploiting alternative raw materials for poultry nutrition in Africa: the case of leaf meals (A. Missohou)

Ayao MISSOHOU

Missohou A., Ayssiwèdé S. B.

Service de Zootechnie-Alimentation, Ecole Inter-Etats des Sciences et Médecine Vétérinaires (EISMV) ; BP 5077 Dakar Sénégal

Abstract

As a solution to the poor competitiveness of the poultry industry, the volatility of conventional ingredients price on international market and feed deficit in rural poultry in Africa, extensive research is being carried out on alternative resources in poultry feeding. Among the many alternative ingredients of interest, leaf meals seem the most promising. They have poor or no competition with human consumption and have quite high protein contents (17 to 36.3%). They also contain some bioactive compounds (phenolic acids, flavonoids, tannins and saponin) that could be beneficial for poultry health. However, their crude fiber (6.7 to 22.5%) and ash (6.7 to 19.3%) contents are high and contribute to their relatively lower energy contents (1800 to 2890 kcal ME/kg). The incorporation of leaf meals in broiler feeds globally lead to poor performances (decrease in growth rate, higher feed intake and feed conversion ratio) as a consequence of the sensitiveness of broilers to high crude fiber and or to antinutritional factors contents. In layers the negative effects of leaf meal inclusion in feed are less pronounced with almost no effect on feed conversion ratio (FCR). The most interesting results of the use of these ingredients in feeding were obtained in indigenous poultry where growth rate, feed intake and FCR were improved even up to 21% incorporation rate in the diet. Leaf meal has also increased yolk, carcass fat, skin and shank colors. The immediate prospects for the use of these alternative ingredients will be in rural poultry or in semicommercial poultry units that employ some degree of on-farm feed mixing.

Key words: Leaf meals, poultry feeding, performance, alternative raw materials, Africa

Genetics by nutrition interaction to optimize gut function

Understanding nutrition by genetics interaction to optimize feed efficiency (S. Mignon-Grasteau)

Sandrine MIGNON-GRASTEAU

INRAE – BOA – 37380 Nouzilly – France

Corresponding author: sandrine.grasteau@inrae.fr

Abbreviated title: Genetics-Nutrition interaction for Feed Efficiency

Summary

Due to the importance of feed cost in the total production cost, feed efficiency is one the most important criterion of selection in chickens. Our review is reviewing the elements of genetic determinism of feed conversion ratio and residual feed intake. In a first part, we show how complex is the determinism of this trait, which depends on many physiological processes as feed intake, metabolism, growth, body composition and behavior. In a second time, we give some elements on the relationship between animal physiology and genetics and microbiota composition and its impact on feed efficiency. In a third part, we discuss about nutrigenomic studies which is now widely used to explain diet effects on feed efficiency. In the end, feed efficiency is the result of a 3-way interaction between animal genetics, diet composition and microbiota.

Keywords: nutrigenetics, nutrigenomics, microbiota, genotype by environment interaction

Introduction

Feed is representing the major part of production cost in chickens, varying between 50 and 70% depending on the type of production. Since decades, feed efficiency has been included in selection schemes, resulting in a spectacular improvement of this trait. In 1947, 5.0 kg of feed were thus required to produce a 1.5 kg chicken in 120 days, whereas in 2000 only 3.5 kg of feed were used to reach 2.2 kg in 35 days. The improvement of efficiency is still continuing now, as illustrated in Figure 1.

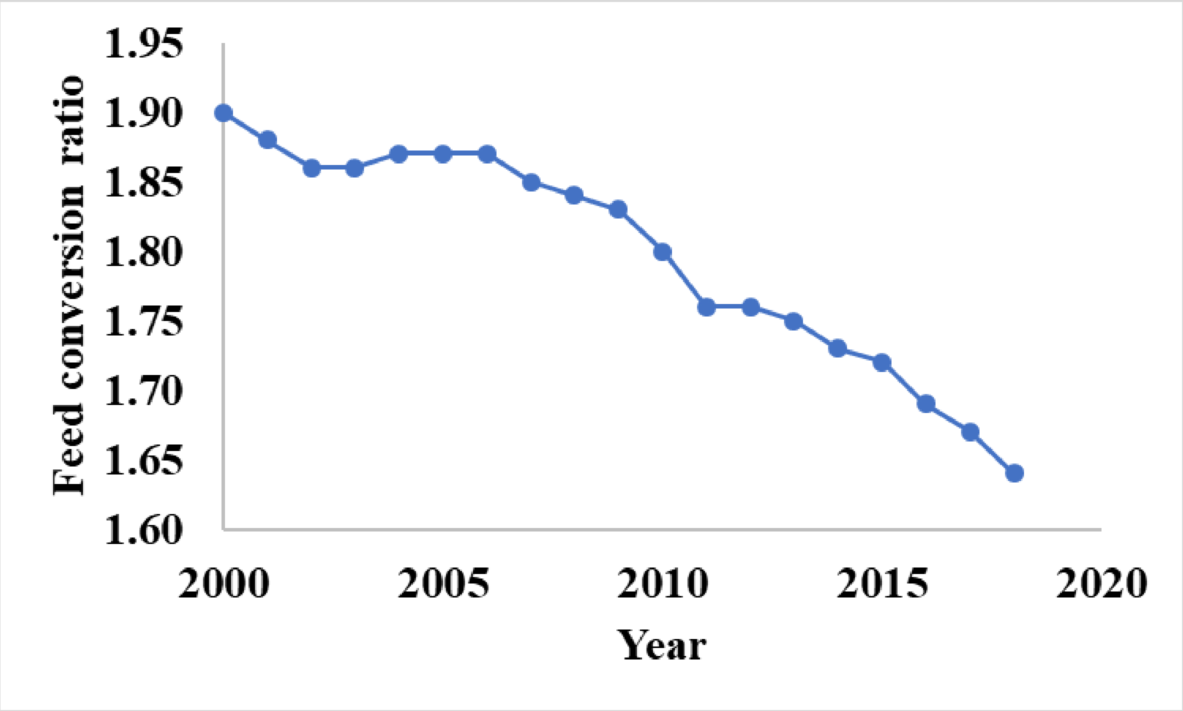

Figure 1. Evolution of feed conversion ratio in rapid-growing broilers since 2000 in France (ITAVI, 2019)

Feed efficiency is usually assessed through two traits: feed conversion ratio (FCR) and residual feed intake (RFI). The first is the ratio of feed intake to weight gain in growing chickens. The second is calculated as the difference between measured feed intake and feed intake estimated by regression on metabolic weight and weight gain. If FCR is widely used for its simplicity, it is also largely criticized as it is a ratio, and that using it in selection schemes makes it difficult to control the relative evolution of numerator and denominator (Aggrey et al., 2010). The question can nevertheless not be reduced to a question of statistical properties of a ratio. Feed efficiency is resulting from multiple physiological processes such as feed intake, digestive efficiency, absorption, animal metabolism, body composition, physical activity…

Moreover, until recently, the conditions of measurement of feed efficiency were different from those encountered during animals' life. To record individual feed intake, animals were placed into individual cages, so that their activity and their social behavior were restricted compared to the real-life production conditions. In these conditions, measurements are usually obtained at weekly intervals in order to limit animal handling, and measures at young age are not available. Several automatic feed stations have been developed in chickens and ducks (Howie et al., 2009; Basso et al., 2014; Guettier et al., 2020), relying on RFID technology to detect the animal presence at the automaton and on scales to record feed and in some cases animal weight. The BIRD-e feed station for example permits a continuous record of animal weight, feed intake, feed efficiency and feeding behavior continuously from 3 days of age (Berger et al., 2021). As animals are reared on floor and in group and that there is no corridor to separate animals from each other in the station, feeding behavior and physical activity are equivalent to those observed in production conditions.

The role of the animal in feed efficiency: nutrigenetics

Due to its economic importance, feed efficiency traits have been included in selection schemes for long time, and many estimates of genetic parameters are already available. Both RFI and FCR have been found to be moderately to highly heritable, ranging from 0.12 to 0.49 for FCR and from 0.18 to 0.49 for RFI (Aggrey et al., 2010; de Verdal et al., 2011; Xu et al., 2016; Yuan et al., 2017; Ye et al., 2020; Li et al., 2021). In addition to direct effects, maternal effects accounted for 1 to 7% of total variability (Li et al., 2021; Berger et al., 2022). Both traits were positively correlated but the correlation varied largely between studies, from 0.21 in Aggrey et al. (2010) to 0.75 in Li et al. (2021). This large variability of estimates of genetic parameters is linked to differences in chicken genotypes, diet composition, conditions of measures (in cage or on floor) and age of animals between studies. Berger et al. (2022) showed for example that heritability of cumulative feed conversion ratio increased with age, from 0.08-0.20 at 5-10 days to 0.27-0.63 at 37-42 days. Age effect is associated with the development of gastro-intestinal tract and with the evolution of body composition with age, mainly the evolution of the ratio of protein to fat deposition, as both do not require the same energy for deposition (Aggrey et al., 2010). Moreover, the diet composition also changes throughout animals' life, which also has an impact on FCR.

Feed efficiency is a complex trait resulting from different processes as ingestion, digestion, metabolism, body composition, physical activity and thermoregulation. All these processes are partially controlled by animal genetics.

At first, regarding the relative importance of weight gain and feed intake in the construction of feed efficiency traits, genetic correlations estimates in the literature show that both RFI and FCR are more driven by feed intake (mean r_g=0.59, range 0.22-0.99 for RFI, µ=0.61, range 0.25-0.94 for FCR) than by weight gain (mean r_g=0.19, range -0.05-0.54 for RFI, µ=-0.26, range -0.80-0.04 for FCR, Pakdel et al., 2005; Aggrey et al., 2010; Howie et al., 2011 ; de Verdal et al., 2013; Xu et al., 2016; Yuan, et al., 2017; Mebratie et al., 2019; Zhang et al., 2020; Li et al., 2021). In these studies, as in the kinetics study of Berger et al. (2022), the genetic correlation between feed efficiency and weight gain tends to have larger values at the end of rearing period, showing that, at later ages, both intake and growth have a similar contribution to feed efficiency.

Digestion has been poorly studied until NIRS helped to predict metabolizable energy or coefficients of digestive use of dry matter, starch, lipids and proteins much faster than with traditional laboratory analyses. Recently, it has been shown that color of the serum of chickens could be used as a biomarker of digestive efficiency, as it was phenotypically and genetically correlated to digestive efficiency (Mignon-Grasteau et al., 2020; Juanchich et al., 2021). This might be associated to the capacity to absorb dietary carotenoids in the intestine (Beauclercq et al., 2019). Provided that animals are challenged with a difficult diet, with high viscosity or hardness for example, digestive efficiency traits are moderately heritable (0.29-0.40 depending on traits, Mignon-Grasteau et al., 2004; de Verdal et al., 2011; Tran et al., 2014). At the opposite, when using diets based on corn and soybean, heritability of digestive efficiency trait is much lower (0.04-0.26), apart from starch digestibility, which has similar heritability values with both types of diets (0.26 with corn, 0.28 with wheat, Mignon-Grasteau et al., 2010). Nevertheless, the high genetic correlation between wheat and corn digestibility traits was highly positive (0.63 to 0.88) showing that selection on a difficult diet would also improve performance on an easy diet. Digestive efficiency in broilers exposed to heat stress also has a moderate heritability (0.33, van Goor et al., 2015). The genetic determinism of digestive efficiency both relies on development and structure of the gastro-intestinal tract and on its operation. In chicken lines divergently selected for digestive efficiency, heavier gizzards and lighter intestines are associated with a better efficiency (de Verdal et al., 2010). Animals with the poor digestive efficiency have larger villi and more goblet cells, especially in the jejunum and ileum, which is an adaptation to compensate their lower capacity to digest (de Verdal et al., 2010). Another striking consequence of selection on digestive efficiency is the difference in transit. The total retention time in the gastro-intestinal tract (GIT) is 48 to 69% longer in the high than in the low line, depending on the size of particles (Rougière and Carré, 2010). The difference of retention time is especially high in the gizzard, letting more time for acid secretion, associated with a lower pH in gizzard of efficient birds (de Verdal et al., 2013). This is favoring the action of gastric enzymes and stimulates the secretion of bile salts in duodenum, improving digestibility of starch, proteins and lipids. A slower transit could also be responsible for differences of degradation of phytic phosphorus in the GIT of laying hens (Sommerfeld et al., 2020).

Metabolism is also part of the genetic determinism of feed efficiency and is associated to metabolic body weight, body composition and thermoregulation. Maintenance cost is a function of metabolic body weight as fasting heat production is proportional to metabolic body weight (Noblet et al., 2015). Estimates of genetic correlations between RFI or FCR and metabolic body weight (0.20-0.25, Pakdel et al., 2005; Aggrey et al., 2010; de Verdal et al., 2011; Rekaya et al., 2013; Xu et al., 2016; Li et al., 2021) confirm that heavier birds need more energy to maintain their body weight. Carré and Juin (2015) estimated that 44% of energy intake of fast-growing broilers could be required for maintenance. This proportion may increase with selection for breast yield as fasting heat production is higher in lean than in fat birds (Noblet et al., 2015). Moreover, as less energy is required for lipid than for protein deposition, animals with a high breast yield and low abdominal fat yield are more efficient than those with low breast yield and high fat yield (Carré and Méda, 2015). The genetic correlations between feed efficiency is much higher with abdominal fat yield (0.37-0.48, N'Dri et al., 2006; Li et al., 2021) than with breast yield (-0.40 to 0.00, N'Dri et al., 2006; Rekaya et al., 2013; Liu et al., 2017). As the relative proportion of lipid and protein in tissue deposition vary with age, these correlations also change with age. For example, Berger et al. (2022) showed that the genetic correlation between breast yield and feed conversion ratio decreased sharply after 20 and 35 d in two different lines of broilers. Finally, thermoregulation processes influence feed efficiency both as animal spend energy to maintain its body temperature (cf. Collin et al., 2022 in this congress for a detailed review) and as feed intake is inducing heat production. For example, depending on environmental temperature (from 14 to 32°C), heat production of *ad libitum* fed birds is 38 to 50% higher than in fasted birds (Koh and Mc Leod, 1999). In a context of global warming, maintaining a sufficient daily feed intake to sustain growth rate without having deleterious effects on animal welfare due to excessive diet-induced heat production can be challenging. The development of automatic feed stations as used in Howie et al. (2011) or Berger et al. (2022) could be a way to find animals with similar total feed intake but feeding mostly during the coldest moments of the day. Howie et al. (2011) showed for example that several parameters of feeding behaviors such as meal duration and size were heritable and positively correlated with feed conversion ratio. This correlation was however line-dependent, the strategy of selection would thus vary between lines.

Energy expenses for physical activity has a negative impact on feed efficiency traits. Luiting et al. (1991) estimated that physical activity would be responsible of 29 to 54% of the difference of heat production between high and low RFI laying hens. Mignon-Grasteau et al. (2017) found a very low genetic correlation between the frequency of moving and starch digestibility (0.09-0.10). However, given that in most studies, birds are reared in cages for the measure of feed intake, the impact of physical activity is probably underestimated. In extensive production with outdoor access, there is thus probably a balance to find between a good feed efficiency and an acceptable level of activity, which is appreciated by consumers.

Positional and functional analyses have identified genes involved in determinism of feed efficiency. A total of 796 QTL are reported in QTL data base (https://www.animalgenome.org/cgi-bin/QTLdb/GG, 24/06/2022). Co-localizations between QTLs between different studies are not so frequent, which probably reflects the complexity of this trait which depends on many different physiological processes, but is also highly dependent on environmental (diet, rearing conditions, temperature), animal (genotype, age, sex) and microbiota factors… Genes identified in QTL/GWAS detection studies cover the various functions of GIT: structure with the pathway of claudins implied in intercellular junctions (eg. *CLDN3*, *EPCAM*), transporters, energy provision (*AGK*, *GTF32*…), immune system (Sell-Kubiak et al., 2017) …Figure 2 illustrates the different functions for which genes related to digestive and feed efficiencies have been identified in chickens selected for their digestive efficiency (Juanchich et al., 2018). By comparing list of DE genes between gizzard and gastroduodenal junction in good digesters and in poor digesters, Juanchich et al. (2019) also found that the dialog between elements of the gastro-intestinal tract was as important as the operation of each tissue taken separately.

If most SNP identified have a moderate effect on feed efficiency, some SNPs identified as affecting feed efficiency explain a high proportion of variability of the trait. For example, Xu et al. (2016) found that a group of 32 SNP explained 53% of the variability of RFI. This region contained genes involved in lipid metabolism, social behavior and immune system. Similarly, Reyer et al. (2015) found that genotype at a given SNP in the *GTF21* gene largely affected FCR at 6 weeks in broilers (from 1.68 to 1.92). This gene is known to affect growth in several species. Grupioni et al. (2017) found an effect of *TNFSF11* on FCR in broilers, but only in males and not in females.

Copy number variation is also suspected to affect digestion and absorption of nutrients in chickens, the CNV of genes as *ANXA10* or *MYLK* being different between fat and lean lines of chickens (Zhang et al., 2014). In humans, the number of copies of amylase has been directly associated to starch digestibility (Urbanski et al., 2021). In addition to SNP effects per se, Sheng et al. (2013) also identified two epistatic interactions between QTLs affecting FCR in slow-growing broilers, between positions GGA3-215 cM and GGA26-40 cM and between GGA20-10 cM and GGA22-40 cM. These two interactions explained respectively 8.1% of variability of FCR between 6 and 8 weeks and 6.4% of the variance of FCR between 8 and 10 weeks.

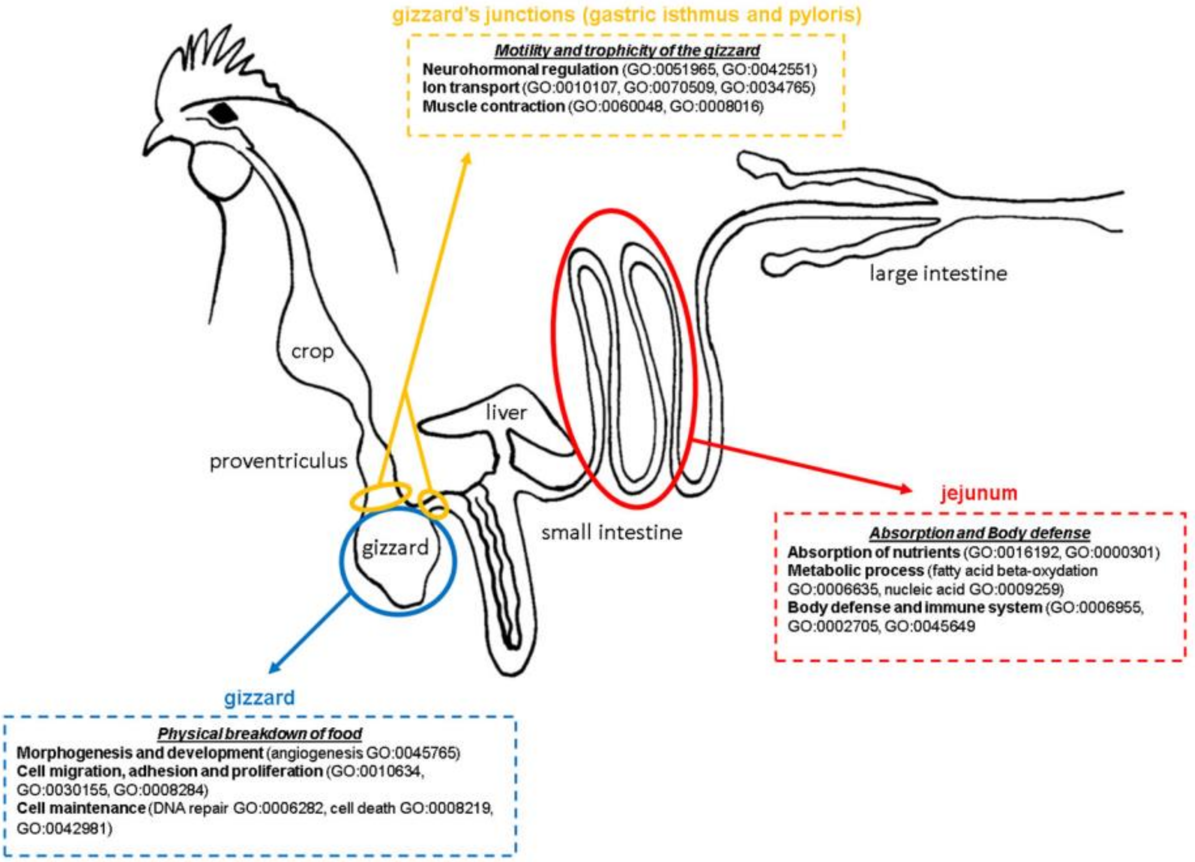

Figure 2. Molecular portrait of the digestive tract in broilers (reproduced from Juanchich et al., 2018).

Interactions between animal and microbiota

The importance of microbiota on digestive and feed efficiency is now assessed by numerous studies. Microbiota can degrade indigestible carbohydrates and transforms them through fermentation as SCFA useable by the host as energy source (Wen et al., 2021). Cecal microbiota is of special importance, as it contributes to nitrogen recycling, vitamin B production, essential amino acids production and digestion of NSP (Borey et al., 2020). Composition of microbiota has been found to differ between animals with contrasted feed and/or digestive efficiencies. In D+/D- lines, Clostridia and E. coli have been associated with a lower digestive efficiency, whereas lactobacilli were associated with better digestive efficiency (Mignon-Grasteau et al., 2015; Borey et al., 2020) and fermentation process of NSP through microbiota is more intense in highly efficient birds (Borey et al., 2020). Microbiota composition is moderately heritable in chickens, which implies that host genetics determines partly the composition of its microbiota, probably to through combined effect of digestive efficiency, transit, feeding behavior and enzymatic secretions, providing more or less favorable environment for bacteria development. More than the number of bacteria of each family, it seems that the equilibrium between families would be the heritable trait. This influence of animal genetics on microbiota composition has been confirmed by the detection of QTL of microbiota composition in several studies in chickens (Mignon-Grasteau et al., 2015; Wen et al., 2021).

More recently, the contribution of microbiota to feed efficiency, defined as microbioability by analogy to heritability has been estimated in chickens and in pigs. The contributions of microbiota to feed efficiency has be found to be of equal importance or even more important than host contribution (Déru et al., 2021; Wen et al., 2021). For example, in chickens, heritability of RFI has been estimated at 0.39, and microbiability at 0.14 in the duodenum and 0.28 in the cecum (Wen et al., 2021). Similar results have been obtained in pigs (Déru et al., 2021).

Interaction between diets and animals: nutrigenomics

Nutrigenomics is defined as the science studying the role of nutrients and bioactive food compounds on gene expression. Nutrients are signals that are detected by the cellular sensor systems that influence gene and protein expression, and thus metabolite production (Müller and Kersten, 2003). Both chemical properties such as carbohydrates, proteins, fatty acids, vitamins and minerals, phytochemicals act as signals (Benitez et al., 2017) and physical characteristics (e.g., particle size, viscosity) can also act as signals. Transcription factors are the main agents through which nutrients influence gene expression (Müller and Kersten, 2003; Benitez et al., 2017). They are mostly found in the superfamily of nuclear hormone receptor, which include bile salts, vitamin D, or retinoic acid receptors (Müller and Kersten, 2003).

For example, Brennan (2015) showed that the addition of mannan oligosaccharides in the diet increased the expression of mucin in the intestine and decreased the expression of genes involved in cell turnover and proliferation, thus leading to an energy sparing. These effects can be on the long-term. For example, Brennan (2015) showed that the mineral content of diet given during the first 96 h of life modulated the expression of solute carrier proteins, a family of transporters essential for the active and passive transport of nutrients in the small intestine in adult birds. Similarly, a moderate restriction in phosphorus at hatch increased the expression of *NaPcoT* in chickens in the long-term, which improves absorption of phosphorus in the intestine. This effect is due to a 43% reduction in the methylation level of the promoter of the gene (Ashwell an Angel, 2010). Addition of methionine in the diet also decrease the expression of *avUCP* in the muscle, thus modifying thermogenesis (Gangula et al., 2018). Addition of some enzymes such as xylanase in wheat-based diets will also increase the expression of insulin like growth factor, thus stimulating protein synthesis and growth (Gangula et al., 2018).

Regarding physical characteristics of the diet, hardness of wheat influences the size of particles present in birds' GIT and thus their capacity to access and digest diet nutrients (Péron et al., 2006), especially for starch, which digestibility increases by 6% when using the Scipion wheat variety (hardness index 5) than with the Baltimore variety (hardness index 75). Viscosity due to the presence of non-starch polysaccharides also limits digestibility, especially for lipids (Péron et al., 2006). Liu et al. (2019) also found that that addition of harder and less accessible particles of corn in the diet stimulated the activity of pancreatic and jejunal enzymes and upregulated the expression of *SGLT-1* and *GLUT-2*, genes involved in glucose uptake in the intestine. In addition to this effects, diet physical characteristics also influence microbiota composition. Indeed, replacing corn by rye as the main cereal in the diet of broilers is known to increase intestinal viscosity, decrease digestibility, letting more undigested material for microbial fermentation leading to bacterial overgrowth in the intestine. For example, coliforms, associated with poor digestibility, are 1.7 to 3.6 more abundant in duodenum and ileum of broilers fed with rye than with corn (Tellez et al., 2014). Retention of calcium and phosphorus in rye-fed birds is also decreased by 27 to 43% (Tellez et al., 2014).

Few studies consider simultaneously variations of the genotype and of the diet, but these studies show how complex the interactions are and how nutritional and selection strategies have to be adapted to each situation. For example, by comparing the response of 3 genotypes of laying hens selected on their immune response to SRBC to 2 different diets, de Greeff et al. (2010) found that genes as the aceoacetyl co-A synthase (involved in the synthesis of cholesterol) was more expressed in jejunum of 2 chicken lines, but less expressed in a third line, when fed with the organic diet. Another example can be found in broilers lines divergently selected for digestive efficiency. Adding xylanase in addition to antibiotics stimulated bile salt production much more in the low efficiency line (+120%) than in most efficient line (+10.7%) thus leading to a larger effect of this additive on lipid digestibility in the low than in the high line (+15% vs +4%, Garcia et al., 2007).

Conclusion and perspectives

Studies of the literature have shown that determinism of feed efficiency is relying both on nutrigenetics (influence of animal genotype on efficiency) and nutrigenomics (effect of diet on expression of animal genes). A third actor, microbiota, is now clearly identified. Feed efficiency is thus finally resulting from a 3-way interaction, or even a 4-way interaction when considering other environmental factors such as temperature or access to open-air for example. These environmental factors are going to take an increasing importance with global warming and social demand for outdoor access in broilers.

Studies considering simultaneously variability in animal genetics and in diet showed that the interactions are complex and that the strategies of animals to adapt to their diets is variable. Considering both diet composition and characteristics, animal genetics should thus be taken into account to propose realistic strategies of selection.

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Amelia CAMARINHA-SILVA

Institute of Animal Science, University of Hohenheim, Germany

Corresponding author: amelia.silva@uni-hohenheim.de

Abbreviated title: Host genetics, nutrition and microbiome

Summary

Livestock gastrointestinal microbiota has a vital role in nutrient digestion, immune system regulation, pathogen inhibition, endocrine activity, and gut epithelium nourishment. Interactions between microorganisms are essential for gut homeostasis, promote the intestinal mucus layer and host metabolism, and affect animal physiology and health. Diet, gender, host genetics, housing, and health status, shape the development and composition of livestock gut microbiota from birth until later stages.

Animal diets provide sufficient nutrients to keep animal performance and health and are sources of energy, proteins, vitamins, amino acids and minerals. Microbiota mediates the breakdown of proteins and nutrient absorption in the gastrointestinal tract. Optimized nutrition is fundamental for livestock production, efficiency, and growth performance, and it has a positive impact on gut microbiota diversity and interactions. The gut microbiota has a cooperative relationship with the host. Host genetic variation contributes to microbiota changes where a single gene can alter bacterial structures. However, it is not yet clear how it occurs and if it influences the parental genome. Host genotype may also modify the microbiota by regulating gut motility and altering the surface of epithelial cells. Microbiota distribution can be used as an explanatory variable for microbial prediction of growth, feed intake, and feed efficiency. Microbiability, defined as the phenotypic variance of the traits explained by the microbiome, might offer innovative breeding possibilities for efficiency traits. A holistic overview of the host-microbiome-nutrition relationships' mechanisms is necessary to deepen our knowledge on diet-host, host-microbiota, diet-microbiota, and microbiota-microbiota interactions and improve animal performance and health.

Keywords: nutrition, microbiome, genetics, host, gastrointestinal tract

Introduction

In the last decades, genetic selection has played an essential role in selecting livestock animals for high growth and reproduction traits. With improvements in husbandry techniques (housing, hygiene, vaccination, etc.), a better understanding of dietary requirements and digestive physiology has enhanced livestock productive performance

(Celi, Cowieson et al. 2017).

Balanced gastrointestinal tract (GIT) functionality is crucial for nutrient digestion, gut homeostasis, interaction with the immune system, and pathogen inhibition by competition for nutrients and adherence to the epithelial layer. A healthy gut colonized by effective gut microbiota is a critical factor towards animal performance (milk yield, body weight gain, egg quality, etc.). Imbalanced gut microbiota affects animal health and host metabolism (Borda-Molina, Seifert et al. 2018).

The livestock industry has a considerable impact on the environment regarding feed production and transportation, and manure management. The use of antibiotics in livestock for health reasons or as growth promoters (where it is still practicable) has a significant impact on society and the environment. The FAO forecast that by 2050, the human population will increase to over 9 billion people, and global meat consumption is estimated to rise by 73% (FAO 2019). It is critical to ensure food availability, nutritional content, discover approaches to improve feed efficiency, nutrient utilization, and methane emission, and look for the microbial mechanisms impacting livestock efficiency while having sustainable livestock production.

Livestock Microbiome

The microbiome includes Bacteria, Archaea, Protozoa, Viruses, their genes, genomes, proteomes, and metabolomes (Marchesi and Ravel 2015). Microorganisms are found in every habitat on earth and are adapted to different physicochemical conditions and the available substrates to grow therein. In the last four billion years, they have co-evolved with their habitats or respective hosts and establish relationships. The microbiome lives in symbiosis with their host and promotes host homeostasis by contributing to energy metabolism regulation, food digestion and absorption, prevention of infections by pathogens, and immune system modulation (Willing and Van Kessel 2010). The availability of substrates and oxygen makes each habitat unique, but in mammals' microorganisms can find a constant temperature that favors their growth. The GIT of the different livestock animals not only differ in their anatomy but also the colonized microbiome, which reflects adaptations to feed source and host genetics.

The use of high-throughput sequencing technologies allows us to deeply investigate the composition and function of the gut microbiome in a cost-effective way. However, they have shown that there is still a long way to describe the microorganisms that are not yet cultured. The most used technique to study livestock microbiota is targeted amplicon sequencing. Microbiota is identified with amplicon sequencing approaches that target 16S rRNA genes, 18S rRNA genes, and ITS regions (Marchesi and Ravel 2015). It has been used to characterize the gut of fowl, pigs, ruminants across their lifespan, gut spatial variation, gender, under a specific dietary treatment and/or feed additive, and regarding host genotype (Borda-Molina, Seifert et al. 2018, Auffret, Stewart et al. 2020, Mon, Zhu et al. 2020, Beaumont, Cauquil et al. 2021). In the last years, some livestock studies used metagenomics to describe the collection of genomes and genes of the gut of fowl, pigs, and ruminants (Huang, Zhang et al. 2018, Xue, Sun et al. 2020, Chen, Zhou et al. 2021). This approach allows the characterization of the bacterial functionality in a specific ecosystem (Marchesi and Ravel 2015). With the decrease of sequence costs in the last years, more studies will be released in the future using this methodology.

The increase of genomes and metagenomes fosters the identification and characterization of expressed proteins by the microorganisms present in the gut at a particular time point. Metaproteomic studies of the livestock animals are now more common in the literature (fowl (Tilocca, Witzig et al. 2016), pig (Tilocca, Burbach et al. 2017), ruminants (Honan and Greenwood 2020) and give more precise understandings of the functions carried out by microorganisms. Metabolomics is now being used to study hundreds of metabolites present in the gastrointestinal tract of fowl (Mon, Zhu et al. 2020), pigs (Beaumont, Cauquil et al. 2021), and ruminants (Xue, Sun et al. 2020).

Despite all ongoing research, meta-omics research is still in its infancy. Livestock microbiome research should move beyond the description of microorganisms, functions, proteins, or metabolites to a causal interaction between the host and the microbiome to differentiate if a shift in the microbiome is a result of host phenotype variation or feed intake and its associated growth parameters.

Feed and its influence on the gut microbiome

The global food demands for meat and milk in the following years need an improvement in food production efficiency (animal and crop) (FAO 2019). Diets are formulated to fulfill animals' requirements to have a good performance and be healthy and include proteins and energy, amino acids, vitamins, and mineral supplements. In the GIT occurs feed digestion and nutrients absorption. A proper formulation of nutrients supplementation is crucial to maintain the functional and structural integrity of the animal tissues (Suttle 2010). Inadequate mineral supplementation of the diet affects the gut microbiome and may cause host disease, resulting in inefficient utilization of natural resources. High supplementation of calcium, nitrogen, phosphorus, and zinc causes their loss in feces and urine (Borda-Molina, Seifert et al. 2018). Malabsorption of macro and micronutrients might increase the passage rate of digesta in the GIT, reducing the available time for nutrient absorption and digestion (Celi, Cowieson et al. 2017).

Some diets also include non-nutritive additives such as antioxidants, antifungals, pigments, and enzymes. Exogenous enzymes improve nutrient availability, animal performance, and overall energy utilization (Kiarie, Romero et al. 2013). They have been recommended as substitutes to antibiotic growth promoters, as they can be a source of substrate for specific bacteria. They improve the intestinal mucin integrity, tight junction integrity, and gut tensile strength and reduce inflammatory responses while increasing substrate to fermentative organisms (Cowieson and Kluenter 2019).

Bacteria can hydrolyze indigestible polysaccharides and carbohydrates that are subsequently fermented into short-chain fatty acids that become a source of energy for the host. Diets highly affect SCFA production, and an imbalanced microbiome decreases SCFA production and enhances gut wall damage and inflammation (Willing and Van Kessel 2010). Diet composition, including feed supplements and nutrients, impacts the development and function of the GIT, passage rates, gut physiology, immune system, pH, and microbiome (Celi, Cowieson et al. 2017). The microbiome synthesizes useful nutrients and proteins and increases the energy gathered from the dietary components. A balanced diet that supports the host with the necessary nutrients during its transition phases (starter, grower, and finisher), age, and stress (transportation, environment, lactation, farrowing, etc.) will improve animal performance and welfare and gut microbiome balance. The nature of the diet and the host-microbiome symbiosis in the gastrointestinal tract of livestock is vital to meet energy and nutrient needs.

Influence of gut microbiome on host genetics

Intensive genetic selection and improvements in health, management, and nutrition have led to extraordinary levels of livestock productivity in the last twenty years (FAO 2019). Quantitative traits are influenced by host genetics and environment. The heritability of the trait determines the response to selection. Furthermore, the introduction of the term holobiont as identification of how important the host-microbiome interactions and their influence on host phenotype are, have received attention in recent years (Theis, Dheilly et al. 2016). Host genetics disturb the microbiome via alteration of gut motility, epithelial cells, and secretions into the lumen. Studies in livestock revealed that host genetics influence the microbiome (Camarinha-Silva, Maushammer et al. 2017). Shotgun metagenomics improves microbiome information. It can be used for animal breeding purposes as a marker for feed conversion ratio, feed intake, feed efficiency, body-weight gain, and minerals utilization (Auffret, Stewart et al. 2020). Understanding the host effect on the gut microbiome requires different approaches, from analysing the gut microbial composition and function to identifying genetic variations that cause microbiome variations. Genome-wide association studies (GWAS) can be utilized to identify chromosomal regions and single nucleotide polymorphisms (SNP) that are associated with the livestock microbiome (Bergamaschi, Maltecca et al. 2020). Thus, developing strategies of hologenomic selection targeting the microbiome and host genome (Weishaar, Wellmann et al. 2020). By applying genomic best linear unbiased prediction (G-BLUP) and microbial best linear unbiased prediction (M-BLUP) methods, the gut microbiota can be used as useful resource of complex trait predictions. Microbiability permits a complete overview of the effect of microbiome on host traits (Camarinha-Silva, Maushammer et al. 2017). By combining information of individuals' phenotypes, genotypes, and gut microbiome, we can further estimate the microbiota effect on the breeding value of a quantitative trait (Weishaar, Wellmann et al. 2020).

Despite the recent years' research, the extent that the livestock gut microbiome is under the host genetic influence needs more examination. Caution should be taken before any selection method can be applied to breeding practice. It would be more appropriate to select the microbiota effect on total merit and feed efficiency and prove its utility under more practical conditions.

Conclusions

There is a need to improve livestock health, welfare, and performance and ensure a holistic overview of the host-microbiome-nutrition interaction. It is crucial to integrate, in a multidisciplinary approach, animal nutrition, including feed supplements, environment, and health challenges with the research in host immunology, physiology, genetics, and the gut microbiome.

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Lifetime management of broiler breeders: Insights from precision feeding research (M. Zuidhof)

Martin ZUIDHOF
4-10M Ag/For Centre | University of Alberta | Edmonton, AB, CA T6G 2P5

mzuidhof@ualberta.ca

Abstract

Broiler breeders carry the genetic potential for fast growth. Without intervention, this can result in obesity and reproductive dysfunction. For decades therefore, managing growth trajectories through feed restriction programs has been key for successful broiler breeding. However, while selection for broiler traits continued, target body weights for parent stock evolved very little, particularly in the pullet rearing period. Recently, we have observed delayed onset of lay and even failure to begin lay under some non-conventional management schemes that reduced fat deposition in pullets. Although these birds were on target body weight and very uniform, there was a lack of physiological uniformity. Ad libitum fed broiler breeder pullets can become sexually mature prior to being photostimulated, so it is evident that in addition to photoperiodic cues, metabolic cues from feeding level and body fat stores also play an important role in the activation of the hypothalamic-pituitary-gonadal (HPG) axis that governs reproduction. Under recommended body weight trajectories modern broiler breeders are on the verge of being too lean. Therefore, optimal lighting and feeding programs are essential for HPG axis activation and reproductive efficiency in modern broiler breeders.

Introduction

After 50 years of selection for broiler traits such as growth rate, lean yield, and efficiency, broilers grow more than 4 times faster, while consuming far less feed (Zuidhof et al., 2014). Broiler breeders must carry the genetic potential for fast growth so that they can pass it on to their broiler progeny. Without intervention, rapid growth can result in obesity, reproductive dysfunction, and reduced health and welfare (Decuypere et al., 2010). For decades therefore, managing growth trajectories through feed restriction programs has been key for successful broiler breeding. However, while selection for broiler traits continued, target body weights for parent stock evolved very little, particularly in the pullet rearing period (Renema et al., 2007). During the entire rearing phase, the amount of feed needed to maintain feed restricted pullets on the target body weight relative to ad libitum-fed counterparts was 57% in a 1978 broiler line, compared with 25% in a 2015 broiler line (Carney et al., 2022). This has resulted in a decrease in fatness that is a key concern with respect to the reproductive success of modern broiler breeders.

Pullet to hen transition

The pullet to hen transition occurs once pullets reach a number of thresholds, including adequate 1) age (hypothalamic maturity); 2) body weight; 3) body composition; and 4) photoperiod. Together, these factors stimulate an endocrine cascade in the HPG axis that results in pubertal development and the start of egg laying (Bedecarrats, 2015). Particularly in feed restricted hens, there is growing evidence that metabolic signalling molecules play a role of growing importance in this cascade (Bedecarrats et al., 2016). Carney et al. (2022) reported that without feed restriction, carcass abdominal fat levels at photostimulation age were similar (6.9% vs. 5.3%) for 1978 vs. 2015 broiler lines, respectively. However, under feed restriction, carcass abdominal fat levels dropped from 2.8% in 1978-line pullets to 0.5% of body weight in 2015-line pullets. Sub-optimal body composition was likely the reason why the most modern strain took an additional 10 days to reach sexual maturity compared to the 1978 and 1995 broiler lines, which likely met all the thresholds for sexual maturation earlier.

In several recent precision feeding studies, we have observed significantly delayed onset of lay and even failure to begin lay (van der Klein et al., 2018). Birds that did not lay eggs were either exposed to conditions that increased gonadotrophin inhibiting hormone (GnIH), which provides negative feedback to the HPG axis. Sexual maturation and egg production were increased when pullets were fed to a higher body weight target. Hadinia et al. (2020) showed that feeding pullets more metabolizable energy during the pullet to hen transition increased the expression of gonadotrophin releasing hormone (GnRH) 2.3-fold and its receptor (GnRH-R1) 1.8-fold with no change in GnIH or its receptor, GnIH-R. Total carcass fat also increased, but carcass protein was not affected. Ad libitum fed broiler breeder pullets can become sexually mature prior to photostimulation. Thus, in addition to photoperiodic cues, metabolic cues from feeding level and body fat stores also play an important role in the activation of the HPG axis that governs reproduction. These results point to either a direct role via metabolites yet to be identified, or indirect role of nutrition (e.g. via signaling molecules from fat tissue) in the onset of lay in broiler breeder pullets.

Management

It is critical to manage multiple thresholds during sexual maturation. Optimal lighting programs that provide no more than 8 hours of light during rearing are necessary to reduce GnIH and its negative feedback to the HPG axis. On the other hand, it seems that introducing positive feedback to the HPG axis by increasing body weight targets can help coax broiler breeder pullets into lay. Feeding management is therefore also important. Higher feeding frequency can increase lean tissue development at the expense of fat stores (Zuidhof, 2018, de Beer and Coon, 2007, Zuidhof et al., 2015, van der Klein et al., 2018). Birds on breeder recommended target body weights with very high uniformity can still be non-uniform physiologically. Under recommended body weight trajectories, modern broiler breeders are on the verge of being too lean. Therefore, optimal lighting and feeding programs are essential for HPG axis activation and reproductive efficiency in modern broiler breeders.

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Early management of broilers: From embryo to the end of starter phase (Z. Uni)

Zehava UNI

Department of Animal Science

Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University, Israel

zehava.uni@mail.huji.ac.il

Abstract

The broiler chicken undergoes major physiological changes during the peri-hatch period, which spans several days before and after hatching, in preparation for life outside the egg. One of the most important changes that occur during this period is a rapid nutritional transition between embryonic and post-hatch feeding. During incubation, chick embryos absorb nutrients from the egg yolk and albumen through the yolk sac tissue, an extra-embryonic tissue which function as intestine, as pancreas, as bone marrow for erythropoiesis and also as the liver. By hatch, the small intestine, the segment of the digestive tract in which the majority of nutrient digestion and absorption occurs, undergoes rapid developmental processes during the peri-hatch period. Today, in our fast-growing modern broilers, the period of embryonic and neonatal development is approaching 50% of the animal life span, therefore there is a need to optimise development and functionality of the YST and the small intestine which support the animal needs for nutrients and energy. Incubation factors such as, temperature, humidity, turning, ventilation, in ovo feeding and delay in feed access affect chick embryo and hatchling digestive and absorptive systems and consequently quality of the hatchlings, BW, breast %, uniformity and FCR.

Introduction

The embryonic and immediate post hatch developmental period represents a significant phase in attaining quality broiler performance at marketing. An efficient transition period from late term embryo to a viable independent chick is necessary for achieving results. Immediately post hatch birds must undergo a shift from egg and embryonic nutrients to exogenous feed.

Under practical conditions many birds have access to feed only 36-72 h after moment of hatch and during this time body weight decreases, intestine and muscle development is retarded. Moreover, as the modern broiler lines are intensively selected for a higher growth rate and increased pectoral muscles, there is an enhanced requirement of chicken embryos for energy and protein, and consequently the imbalance between requirement and reserves of nutrients stored within eggs may limit maximal growth and development of chicken embryos. Accordingly, some of the challenges faced by broilers chicks include weakness, reduced feed intake, impaired growth, susceptibility to disease, and mortality. These symptoms may be due to limitations in some nutrients and energy and to immature digestive system unable to reload depleted energy reserves from consumed feed.

Processes that occur during the critical period of last days of incubation to the end of the starter phase mediate the transition from embryo to independent chick. During this period, chicks make the metabolic and physiological transition from egg nutrients (i.e. yolk content and amniotic fluid) to exogenous formulated starter feed.

This transition period (from embryo to chicken) is accomplished by functional transition between two different digestive organs: First, the yolk sac tissue (YST) which operates during the embryonic phase and is responsible for the abilities of digestion and absorption yolk content. Second, the small intestine which started its absorption and digestion functionality few days before hatch, then rapidly mature, exhibiting enlargement of digestive and absorptive surface by elongation of villi length, elevation of enterocyte number cell and elongation of enterocyte's microvilli. All leads to elevated intestinal capacity for digestion and absorption by the young chick.

Today, in our fast-growing modern broilers, the period of embryonic and neonatal development is approaching 50% of the animal life span. Therefore, methods and approaches that will promote the developmental functionality of the digestive organ, in the pre-post- hatch period, will support the nutritional needs of the hatchlings during this transition period.

In this presentation, I will cover the development and functionality of the digestive organ in the embryo i.e. the yolk sac tissue (YST) that covers the yolk, and the developmental functionality of the intestine in the starter phase. I will show that Incubation temperature has a major effect on YST functionality and therefore on quality of hatchlings. In addition, the benefit of early feeding methods -in ovo feeding at E17 and early feeding immediately post hatch will be presented.

The importance of an optimally-functioning yolk sac tissue (YST) for high quality hatchling

During incubation, most of the yolk nutrients are utilized via the yolk sac tissue (YST), an extra-embryonic tissue that envelopes the yolk (1) Prior to the development of fully functional organs, the embryonic chick must rely upon the yolk content to provide nutrients as well as all of its essential metabolic functions.

Findings from Wong and Uni laboratories (2) shows that the YST acts as a multifunctional organ: As the intestine for digestion and transport of nutrients and lipids to the developing embryo, as the bone marrow for erythropoiesis, as the liver for synthesis of plasma carrier proteins and carbohydrate metabolism. Also, YST function as the thyroid for regulating metabolism and as the immune system for production of host defense peptides. Late in embryogenesis, the YST begins to degrade as the chick develops functioning organs. Thus, although the YS tissue is a transitory organ, it is most essential for the growth, development and health of the developing embryo and the hatchling. During its development, the YST projects villi structures into the yolk content which increase the surface area available for absorption, similar to the villi of the digestive tract. The villi are comprised of endodermal epithelial cells (EEC's) that mediate the transport of nutrients from the yolk content to the blood circulation of the developing embryo (3,4,5,6).

The critical importance of the YST to the emnryonic development in the hatchery It is known by now that the YST serves as the main site of digestion, absorption and nutrient transfer from the yolk content to the developing embryo. Research showed that the YST perform "intestine like" functions by producing digestive enzymes and expressing nutrient transporters (7, 8, 9). Furthermore, studies are pointing towards the conclusion that the YST is a metabolic organ capable of multiple functionalities since it synthesizes bile acids, which are critical for yolk lipid digestion (10). It accomplishes "liver like" functions such as glucose synthesis from non-carbohydrates precursors (gluconeogenesis) as well as glycogen synthesis (11) and regulation of thyroid hormones (12). The YST also demonstrate roles in hematopoiesis and bone marrow development, as it serves as the only source of cells for primitive erythropoiesis and the main niche and source of cells for early definitive erythropoiesis (1, 5). A whole transcriptome analysis (13) showed temporal large-scale expression of genes associated with the metabolic processes that are occurring in the YST from E13 to day of hatch (E21) and demonstrated the various functions of the YST that are mentioned above.

The YST along with its remaining yolk content are referred as the residual yolk sac (RSY). From embryonic day 19 (E19), the RSY begins to be internalized into the embryo’s body cavity and provide the peri-hatch embryos and hatchling with nourishment (1, 14). Appropriate utilization of yolk (i.e. low residual yolk weight relative to embryo weight) and also proper amount of nutrients stored in the yolk sac (i.e. glycogen, glucose, fatty acids and peptides) are key factors for high quality embryos and hatchlings (11, 15). These factors may possibly be affected by incubation conditions. It is well documented that inadequate temperature reduces yolk utilization and leads to low quality embryos and hatchlings which impact broiler performance (16, 17,18).

Incubation temperature changes YST functionality, subsequently affecting hatchling quality

A study in our lab (19) emphasizes the importance of incubation temperature on YST functionality for yolk utilization and for metabolic processes that affect embryos and hatchlings quality. Our findings show that 1.5 °C deviation, from the 37.8 °C optimal incubation temperature, resulted in altered expression of YST genes. There was alternation in pattern of expression of genes encoding for LRP2 (a lipoprotein receptor), ApoA1 (an Apolipoprotein, indicator of lipoprotein metabolism), PepT1 (an oligo-peptide transporter), GYS2 (glycogen synthase) and FBP1 (a gluconeogenesis regulator gene) which are responsible for glucose and glycogen synthesis and for absorption and digestion of yolk lipids and peptides. Accordingly, residual yolk weight towards hatch (RSY%) is changed and the chick embryo has nutrients limitations.

In addition, the study also showed altered expression of YST genes encoding for thyroid hormones (THs) regulators (TTR, DIO1 and DIO2) during incubation period. Since THs are responsible for various processes, including regulation of heat production and mobilization of glycogen reserves (20), changes in their expression, may also affect embryo and hatchling quality. The results point toward the YST as a major multifunctional metabolic tissue during incubation, which has responsiveness and sensitivity to incubation temperature.

Incubation temperature has an effect on gene expression in the YST, thereby affecting yolk utilization and yolk sac nutrients resources leading to either poor or improved quality of embryos and hatchlings. Based on the findings it is proposed to find more environmental and nutritional factors that can optimize YST functionality during incubation period and will lead to improved hatchery practice and production of better-quality embryos, hatchlings and growing chickens.

Early feeding

The need for early nutritional methods is because currently the period of embryonic and neonatal development is approaching 50% of the productive life of modern broilers. Therefore, the embryonic and immediate post hatch developmental period represents a significant phase in attaining quality broiler performance at marketing. Due to increased metabolic rate of today's fast-growing embryos, the embryonic nutrient reserves may be limited or insufficient and some nutrients are depleted in the prenatal period. Moreover, chicks are commonly fasted for the first 36 to 72 h post hatch because of the logistics of commercial production. Fasting during the pre-post-hatch period leads to low quality hatchlings, which exhibit retarded BW, delayed intestinal development, and lower pectoral muscle weight. Therefore, in ovo feeding (IOF), which is the administration of exogenous nutrients into the amnion, enhance egg nutriture and early growth performance by improving pre-hatch chicken embryo energetic and nutritional status, driving functional maturation of the small intestine and the bones. Experiments from various research groups worldwide exhibit the benefits of in ovo feeding method on BW, FCR, breast meat yield and other important production measures (21). A 0.5 ml sterilized solution injected to the amnion at E17.5-E18 which includes macro-nutrients, salts, minerals and vitamins, elevates their levels and availability for the embryo and support the development of the intestine and other critical organs. This has a great importance in case of sub-optimal nutrients/ minerals/vitamins levels, which may limit the genetic potential of growth of critical organs.

Supplementing the amnion fluid with appropriate nutrients (by in ovo feeding) is a novel way to feed critical dietary components to embryos and ‘jump-start’ development of the chick. In-ovo feeding technology has established a new science of perinatal nutrition that will open opportunities for greater production efficiency and animal welfare.

This technology will probably revolutionize early nutrition and incubation practices. The challenge is to overcome the IOF formulation and delivery constraints so it can be commercially applied to achieve a better return on investment. It is predicted that as it happened with in ovo vaccination, in ovo feeding may be commercially practical.

In ovo feeding and intestinal development (cell types, microvilli, digestive enzymes and transporters)

In the first two days' post hatch chick the small intestinal mucosa appears to be immature and not fully developed. However, later on, from day 3 to day 10, the intestinal mucosa exhibit organization and establishment of the crypt region, a several-fold increase in villus height and area, an increase in the number and polarity of enterocytes and maturation of the goblet cells, which are capable of producing both acidic and neutral mucins.

The immediate post-hatch period is critical for intestinal development. Decreased development was found when chickens were fasted for 36 to 48 h post-hatch. This "fasting" condition is a common situation in the poultry industry. Since chicken embryos have a wide "hatching window", commercial hatcheries do not remove birds until the maximum number of eggs have hatched; thus, chick age at exit from the hatchery averages more than 1 day. Hatchery treatments such as sexing, vaccination and transport to farms result in an additional time lag before birds receive first access to food and water. Thus, most chicks are fasted for 48 h or more before their first access to feed. The concept that, meanwhile this process, the yolk sac can maintain the hatchling, until stable feeding becomes available, is not proper for the current fast-growing breeds. It has been shown that 36 to 48 h of fasting immediately post-hatch decreases enterocyte number, crypt size, the number of crypts per villus, crypt proliferation, villus area, and rate of enterocyte migration, goblet-cell size and mucin dynamics. This withholding of feed also results in a decrease in growth at an early age and lower body weight (BW) and proportion of breast muscle at marketing.

Since access to feed soon after hatch is critical for the development of the intestine and its digestive capacity there is a need to feed the hatchlings as soon as they hatched. A large body of knowledge shows that “feeding” the embryo 3 days before hatch (by in ovo feeding methodology; US patent Uni and Ferket 2003) accelerate enteric development and its capacity to digest nutrients. By injecting an isotonic in ovo feeding (IOF) solution into the embryonic amnion, the embryo can naturally consume supplemental nutrients orally before hatching. In ovo feeding, “jump-start” and stimulate intestinal development to begin earlier than would otherwise occur after. The sooner the intestine achieves functional capacity the earlier the animal can utilize dietary nutrients, absorb minerals and vitamins and support the development of critical organs (skeleton, immune system, breast muscle).

In ovo feeding clearly advances the digestive capacity and development of intestine of the pre-hatch embryo by about 2 days at the time of hatch: Using scanning electron microscopy, Bohórquez et al. (2008) observed that in ovo feeding significantly increased functional maturity and mucus secretion of goblet cells of villi of ileum and ceca of turkey poults. Advanced morphometic development of the intestinal tract (22,23,) and mucin barrier (24) was observed; enhanced expression of genes for brush boarder enzymes (sucrase-isomaltase, leucine aminopeptidase) and their biological activities, along with enhanced expression of nutrient transporters, SGLT-1, PEPT-1, and NaK ATPase (25,26 was presented. In ovo feeding may help improve the colonization resistance of enteric pathogens of neonatal chicks: Recent work in our lab (27,28) examined the effect of in ovo administration of mannanoligosaccharides (MOS) on chicken small intestine development and reveled positive effects of in-ovo administrated MOS on intestinal maturation and enterocyte development 24 h after in-ovo administration.

Modulating cellular proliferation and differentiation in the small intestinal epithelium of peri-hatch chicks by early feeding strategies

Recent publication from our lab (29,30) showed that in ovo feeding with specific amino acids modulate proliferation and differentiation of multipotent cells in the small intestinal epithelium of peri-hatch chicks, in scope of increasing enterocyte differentiation and maturation for improved nutrient absorption. The effects of immediate post-hatch feeding (early feeding) on crypt and villi intestinal enterocyte cells (IEC) populations were examined during the first 10 d post-hatch, in comparison to a 24 h delay in first feeding (delayed feeding), commonly practiced in poultry farming. Specific markers for IEC populations, such as Lgr5 (stem cells), Sox9 (multipotent cells), PCNA (proliferating cells), PepT1 (enterocytes) and AB-PAS mucin staining (goblet cells), were visualized and quantified in-situ, by RNAscope® in-situ hybridization, immunofluorescence and histochemical techniques. Results showed that compared to delay feeding, early feeding promoted proliferation and differentiation of multipotent cells in the crypt epithelium. This resulted in consistently higher quantities of differentiated enterocytes and goblet cells in the villus epithelium throughout the first 10 d post-hatch. The conclusion from this study is that the timing of first feeding determines the rate of IEC proliferation and differentiation.

First feeding can also be administered to the small intestine before hatch by in-ovo feeding (IOF). Moreover, when IOF of two mitogenic amino acids, glutamine (IOF-Gln) and leucine (IOF-Leu), as well as NaCl, followed by early feeding post-hatch showed that IOF of all nutrients promoted the development of the embryonic small intestinal epithelium within 48 h, through increased proliferation of multipotent IECs and advanced zonation of Lgr5-expressing stem cells to future crypt niches. Characterization of microvilli development by scanning electron microscopy (SEM), in Ultrastructural micrographs of enterocyte apical membranes, showed that microvilli develop rapidly during the peri-hatch period, by increasing in length and density. During the first 3 d post-hatch, microvilli generate a functional brush border that dramatically expands the small intestinal surface area, before villi structures reach morphological maturity through IEC proliferation and differentiation.

The effect of IOF-Glutamine (IOF-Gln) and IOF-NaCl on enterocyte morphological and functional maturation was examined in comparison to a non-IOF control. Enterocyte border visualization by E-cadherin immunofluorescence revealed that only IOF-Gln elicited early onset of enterocyte growth, and their apical microvilli were significantly longer throughout the peri-hatch period, as visualized by SEM.

IOF-Gln also promoted functional maturation of enterocytes, through increased expression of brush border nutrient transporters PepT1 and SGLT1 and tight junction genes TJP-1, TJP-2 and Occludin during the peri-hatch period.

Integration of the effects of IOF-Gln on enterocyte differentiation and maturation hinted at a possible enteroendocrine involvement. It was also shown thatthe effects of IOF-Gln on enterocyte differentiation and maturation may be attributed to L-cell stimulation.

It can be concluded that initial nutrition stimulation in embryo or in hatchling is a robust driving force for IEC proliferation and differentiation. When applied before hatch through IOF, glutamine can promote enterocyte differentiation and morpho-functional maturation, and activate an enteroendocrine system throughout the peri-hatch period. Therefore, early feeding strategies can be used for modulating proliferation and differentiation of IECs in peri-hatch chicks for improving nutrient absorption during this critical pre-post hatch phase.

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Genetics by environment interactions and epigenetics

Epigenetics as a mediator of genome x environment interactions (F. Pitel)

Frédérique PITEL

F. Pitel (1), I. David (1), J. Demars (1), S. Mignon-Grasteau (2), S. Lagarrigue (3), T. Zerjal (4)
(1) GenPhySE, Université de Toulouse, INRAE, ENVT, F-31326, Castanet Tolosan, France
(2) INRAE, Université de Tours, BOA, F- 37380, Nouzilly, France
(3) PEGASE UMR 1348, INRA, AGROCAMPUS OUEST, 35590, Saint-Gilles, France
(4) GABI, INRAE, Univ. Paris-Saclay, AgroParisTech, 78350, Jouy-en-Josas, France

Corresponding authors: frederique.pitel@inrae.fr, tatiana.zerjal@inrae.fr

Abbreviated Title: G×E and epigenetics

Summary

Epigenetic mechanisms, defined as heritable changes in gene function that cannot be explained by changes in the DNA sequence, may play a role in the genotype × environment interaction. Here we briefly show how epigenetics can interact with the environment, how these mechanisms are linked to genetics, and to what extent they can mediate the G×E interaction. The classical genetic selection model decomposes the phenotype into genetic and environmental effects, poorly taking into account their interaction. But this decomposition may be improved. Given the constraints faced by livestock breeding today, especially due to climate change, a better understanding of the epigenetic mechanisms governing the genome's responses to changing environments could provide new routes for improving selection for a wide range of traits.

Key words: Epigenetics, Genotype, Environment, GxE interaction, Breeding

Introduction

Animals in livestock systems face multiple constraints during life and have to adapt to different challenges such as changes in the rearing environment, feed transitions, climate variations, or diseases. These potentially stressful events can have a strong effect on production, health and welfare, the magnitude of which varies among individuals, particularly according to their genotype (Nauta *et al.* 2006, Cardoso and Tempelman 2012). Over the last decades genetic selection has contributed to greatly improve livestock performance: in poultry breeding the increase in performance has been remarkable both in broilers and layers (see (Aggrey *et al.* 2020)). The genetic selection model considers that phenotype is decomposed into genetic (G) and environmental (E) effects, and eventually the interaction between the two (G×E). We know nowadays that this decomposition of components affecting phenotypes is over simplistic. G×E interaction has been known for a long time to influence phenotypes, but the classical selection methods were not able to take it precisely into account. Furthermore, breeding conditions were often quite standardised and its intra-breed impact was not so important. However, with the agro-ecological transition due to climate change, breeding systems are evolving. For example, there is a tendency to lengthen the production careers of females (dairy cattle, layers), the animals being of varying ages and more exposed to varying environments during their lives. In addition, the conditions in which animals are reared may be less uniform with sometimes extreme variations, for example heat waves. So, understanding the G×E component could be important to be able to breed better adaptable genotypes.

There are growing evidences that the environment experienced by the animal may also induce long-term modifications of non-genetic inherited factors that have an impact on phenotype and thus should be taken into account in the phenotype decomposition (see David *et al.* 2019a). Indeed, the environment contributes to a large fraction of the variability of complex traits, notably through epigenetic phenomena: the activation and inactivation of genes that underlay expression variation are partly regulated by epigenetic marks, in part triggered by the environment. These phenomena are therefore part of the mechanisms underlying Genotype × Environment interactions.

1 - Epigenetics

What is epigenetics?

Several definitions of epigenetics coexist, the most widely used being "The study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence" (Riggs *et al.* 1996). Epigenetics can be considered as one of the conductors that contribute to regulate gene expression, for example by adding small chemical modifications to the DNA. In order to enter the restricted space of the cell nuclei, DNA is compacted in the form of a double helix surrounded by nucleosomes, small units made up of particular proteins, the histones. These proteins can undergo numerous chemical modifications (acetylation, methylation, etc.) which contribute to increase or decrease the accessibility of the DNA molecule to the cellular machinery of gene expression. The DNA itself can be modified by the addition of a methyl group on certain bases (mostly cytosines in a 'cytosine - guanine' environment, CG, in vertebrates). This multitude of chemical tags are all epigenetic marks that act as turning genes on or off and controlling the level of gene expression. Transcription of non-coding RNA interacting with DNA is an additional mechanism capable of regulating gene expression. All these marks constitute the epigenome, which is established during the embryonic development when cells become specialized, and differs according to cell type and tissue specificity. Epigenetic marks are involved in various mechanisms such as cell differentiation, inactivation of an X chromosome in female mammalian cells, parental genomic imprinting, or differentiation between monozygotic twins.

These marks are partially reversible, which makes them an effective lever for supporting the genome's response to environmental variation (Feil and Fraga 2011).

What links between epigenetics and environment?

As well as the genome of an organism influences its response to the environment, the environment can also modify the expression of genes through epigenetic mechanisms. Biotic disturbances (changes in the quality or quantity of nutrients, hormone levels...) or abiotic stresses (heat waves...) during prenatal development can induce modifications of these marks, ensuring a dynamic regulation of gene expression (Feil and Fraga 2011). These same disturbances during the productive life, notably for species with long production careers, can also change epigenetic marks, resulting in phenotype variation during the animal lifetime. These epigenetic marks are then partially transmitted during cell division and, when maintained, represent a persistent chemical memory of previous disturbances (Skinner 2011).

In plants, the importance of epigenetics in responses to environmental variations is well established, for instance with the vernalization process (Friedrich *et al.* 2019). In animals, epigenetic modifications can also mediate the variation of gene expression due to environmental effects such as nutrition, (see Langley-Evans 2015, Chavatte-Palmer *et al.* 2016). A recent study in capelin (*Mallotus villosus*), a marine fish, showed that variations in reproductive strategy triggered by the environment (different life histories, in particular low and stable temperature vs high and variable temperature) are more likely governed by epigenetic changes than by genetic modifications (Cayuela *et al.* 2021). Other famous examples of environmentally induced epigenetic mechanisms are the fate of bee larvae that evolve into queens or workers depending on the diet they receive, and the sex determination in turtles, which depends on the temperature during egg incubation (Ge *et al.* 2018, Wojciechowski *et al.* 2018). In the well-known viable yellow agouti model in mice, it has been shown that dietary supplementation with genistein, a phytoestrogen known to modify DNA methylation, can modify phenotypes by altering the epigenome (Dolinoy *et al.* 2006).

In poultry, epigenetic analyses of environmental influences on phenotype are scarce, but several examples can be found in studies about nutrition or thermal manipulation during early life or incubation (see Buyse *et al.* 2020). Histone post-translational modifications in the chicken hypothalamus have been shown to be impacted by embryonic thermal manipulation, and may be ultimately involved in the thermal acclimation of adult birds (David *et al.* 2019b). Recent examples demonstrate the association between DNA methylation and incubation conditions (Corbett *et al.* 2020), detrimental early life conditions (Pértille *et al.* 2017) or chronic stress (Pértille *et al.* 2020). These examples highlight the fact that the exposure to unfavorable specific environmental conditions lead to epigenetic changes which could serve as stress related biomarkers, but they do not contribute to understand if these epigenetic changes are the causative factors of phenotype variability. Of note, favorable environments, as for example exercise training in human, can also trigger changes in epigenetic marks that lead in particular to a better protection from diseases (see Denham 2018, and below).

Part of this environmental memory may be transmitted along generations, a phenomenon whose extent is debated but which may improve genetic selection models if taken into account. The actual contribution of epigenetics to phenotypic variation is scarcely assessed. Studies in quail have estimated epigenetic heritability for body weight and egg quality traits, showing that epigenetic heritability was 0.10 for body weight at 7 days of age, and close to zero for the other traits studied (Paiva *et al.* 2018a, Paiva *et al.* 2018b). The authors concluded that including the epigenetic effect in the animal model helped to explain the residual and non-Mendelian variability of initial body weight.

To know the role of genetics and epigenetics in the construction of phenotypes would allow to improve models used in genetic selection. Similarly, to know the effect of the parental environment on the offspring performance and health will contribute to improve farming systems in order to favor epigenomes that have positive effects on performance and adaptive capacity.

While, as illustrated above, both genetics and epigenetics contribute to phenotypes, they are not two completely unrelated processes.

What links between epigenetics and genetics?

It is now well established that DNA polymorphisms can affect epigenetic marks (Bell *et al.* 2011, Do *et al.* 2017). For example, deGoede et al recently showed that 67.3% of all human annotated lncRNA genes had their expression significantly associated with at least one DNA variant in at least one tissue (de Goede *et al.* 2021). Similarly, up to 80% of the variation in DNA methylation can be explained by the genotype (Gertz *et al.* 2011). A large sample-sized study in human demonstrated the association of long-range regulation of CpG methylation with genetic polymorphisms (Lemire *et al.* 2015). Do et al. have reviewed many studies revealing the existence of a large genetic-driven epigenetic variability (Do *et al.* 2017). This can affect DNA methylation, detected through allele-specific methylation or meQTLs (QTLs responsible for CpG methylation level variability), and chromatin conformation, identified by allele-specific chromatin accessibility. One mechanism explaining this influence of genotypes on epigenetic state is the fact that DNA sequence at specific binding sites can affect the binding of transcription factors, some of these transcription factors being able to modify the DNA methylation level nearby (Feldmann *et al.* 2013).

On the other side, mutation rates can also be affected by epigenetic states: CpG dinucleotides have a mutation rate about 12 times higher than other transition types, because methylcytosines are hypermutable (Sved and Bird 1990), and CpG content may even affect non-CpG mutation rate (Walser and Furano 2010). More generally, associations between chromatin structure and mutation rates have been reported (Makova and Hardison 2015). Thus, environmental exposures could not only select, but also play a direct role on DNA polymorphisms, even if the currently published studies rely on correlations, non-demonstrative for a causative action. These phenomena, when triggered by the environment for generations through epigenetic marks leading to genetic modifications, have been called the "epigenetically facilitated mutational assimilation" (Danchin *et al.* 2019).

Having observed that epigenetic mechanisms are linked to the environment and to the genetics of an organism, the question is to what extent they can mediate the G×E interaction.

2 - Genotype × Environment interactions from an epigenetic perspective

When is "G×E" referred to?

Genotype × environment interactions determine how individuals with different genotypes will respond differently to different environments. Environments can be different in term of biotic (hormone concentration during development, disease, etc) or abiotic (temperature, exposure to chemical contaminants, etc) factors. These interactions may modify the ranking, according to performances, of different individuals when exposed to different environments (Haldane 1946) (Figure 1).

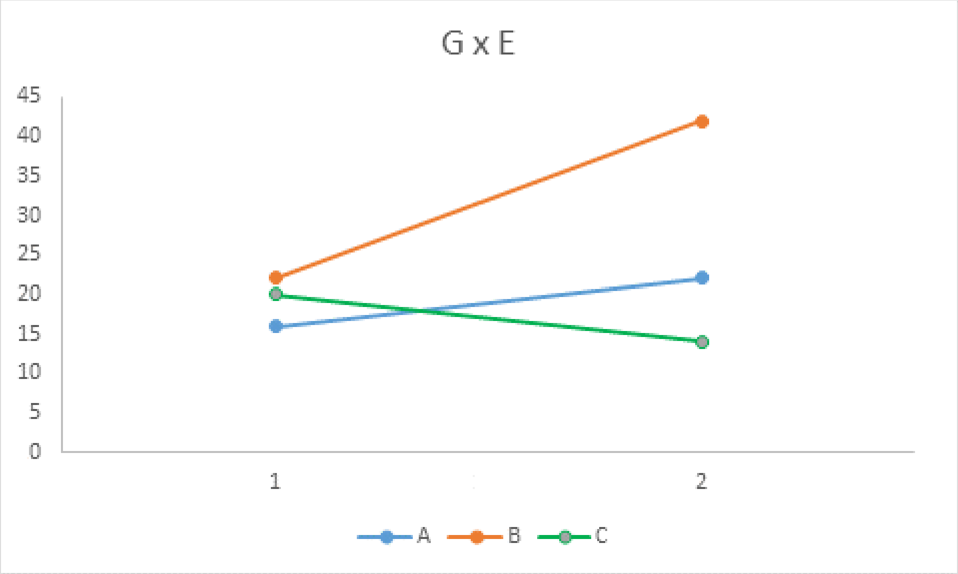

Figure 1: Example of genotype by environment interaction analysis

Arbitrary unit for the phenotypic value of a quantitative trait showing G×E (3 genotypes: A, B, C in 2 environments: 1 and 2)

G×E interactions have been thoroughly studied in cattle (e.g. Hayes *et al.* 2009, Bernabucci *et al.* 2014). While re-ranking of sires in dairy cattle may be limited (Calus and Veerkamp 2003), G×E interaction was shown to affect milk production traits (Hayes *et al.* 2003). In beef cattle, bull re-ranking was shown to occur mostly in restrictive environments, highlighting the importance of evaluating the consequences of a mismatch between selection and production environments (Corrêa *et al.* 2010, Santana *et al.* 2013). In breeding programs such as those encountered in pig and poultry, a loss in genetic gain due to G×E interaction can also be observed (Mulder and Bijma 2005). G×E interaction has been observed for several traits both in broiler (slow-growing, N'Dri A *et al.* 2007) and laying hen (Mathur and Horst 1994) productions. In laying hens, QTL × diet and QTL × age interactions were shown to affect different production traits (Romé *et al.* 2015).

As described by Lillehammer et al (Lillehammer *et al.* 2009) in dairy cattle, genetic polymorphisms can cause this G×E interactions. Some alleles may affect both production and environment susceptibility, and those maintaining or improving production while reducing environmental sensitivity could be good candidates for marker-assisted selection for robustness. Other genes may contribute to the change in animal ranking under various environments. In this case, the selection for the improving allele in one environment may cause a loss in performance under a different environment. An example of this mechanism involving different genes in different environments is found for feed efficiency in poultry (Mignon-Grasteau *et al.* 2010): with an easily digested corn-soya diet, the genetic component of digestive efficiency will be little involved in the variability of feed efficiency, whereas with a diet that is difficult to digest, this digestive component will be more important, and will involve different metabolic pathways, and therefore different genes.

It has been shown that most genes have a positive correlation between general production and environmental sensitivity with the consequence that environmental sensitivity increases with selection for high performance (Rauw and Gomez-Raya 2015). This detrimental link may be due to trade-off between functions (Friggens *et al.* 2017).

Breeders are thus faced with the challenge of how to manage these possible changes in rank: is it preferable to select the best animals in a given environment taking the risk that their performances will be highly modified if environmental conditions are changing or is it preferable to select animals with more modest performances but stable over a wide variety of environments?

In the case of poultry, while the importance of G×E is quite limited for the selected pure lines that are kept under a standardized housing system, it becomes relevant for the crossbred commercial lines reared for human consumption, as commercial breeding companies operate in a global context and distribute their breeding stock worldwide. This is even more accurate for alternative productions (organic, label), which are reared with an outdoor access, whereas the breeding stock is reared in closed pens. The quantification of the interaction between genetics and environment becomes therefore necessary to improve the prediction accuracy of the models across different environments and to supply breeding stocks that are able to express their optimal performances under a wide range of production environments. To achieve this, one strategy would be to account for field performance of crossbred birds in pure line genetic improvement (Esfandyari *et al.* 2016, Duenk *et al.* 2019). An alternative way can be to take advantage of the pyramid structure of breeding (Hiemstra and Napel 2013): pure lines are bred in a controlled environment, and parents of the commercial offspring are bred in the different places/climates where the commercial animals will be bred. The adaptation of the animals for one generation should lead to better performance of the final offspring. Unravelling the impact of the environment on phenotypic variation, largely mediated by interactions between genetic and non-genetic components, is therefore necessary to reach the ultimate goal of improved prediction of an animal’s phenotype based on genotypic and environmental information.

The interaction between genome and environment is thus a phenomenon that has been known for a long time, but studies trying to decipher the molecular mechanisms underlying G x E interactions are scarce.

What molecular mechanisms underlying G×E?

Observation of allele-specific responses to different environments

Several studies have already demonstrated the existence of genomic regions that respond differently to the environment depending on the allele carried.

The existence of environmental "susceptible" or "resistant" alleles is well illustrated by the influence of physical activity on the effects of risk alleles of the fat mass and obesity associated gene (*FTO*) on obesity: the effects of these detrimental alleles are significant only in people with low physical activities (Rampersaud *et al.* 2008).

By using different cell types and different treatments, Moyerbrailean et al. identified 215 genes whose ASE (allele-specific expression) was triggered by the environment, half of which had been identified by GWAS as associated with complex traits (Moyerbrailean *et al.* 2016).

The concept of "response-eQTL" (reQTL) has been developed to qualify eQTLs whose effects depend on the environment, for instance differing according to different immune stimuli (Kim-Hellmuth *et al.* 2017). Kim-Hellmuth et al. identified 417 reQTLs in human monocytes that were differentially responding to various immune stimulations, thus demonstrating the interaction between infectious stimuli and genetic predisposition to diseases (Kim-Hellmuth *et al.* 2017). Alasoo et al. detected reQTLs and "conformation accessibility" QTLs in human macrophages in response to stimulation by IFNγ and/or Salmonella, with a probable impact on the binding of cell-type-specific transcription factors (Alasoo *et al.* 2018).

These allele-specific responses may be the result of environment-specific gene expression or of allelic effects that vary from one environment to another (Rauw and Gomez-Raya 2015). Studies trying to decipher the underlying molecular mechanisms are rather scarce, notably because it requires a larger number of individuals to highlight an interaction between two factors than to demonstrate the effects of a single factor on a given trait. Nevertheless, a growing number of studies have shown that these interactions can be triggered by epigenetic mechanisms, as illustrated by the examples below.

G×E mechanisms observed at specific genomic positions

Allele-specific DNA methylation as a mechanism of genotype × environment interaction was demonstrated in mice. Holland et al. showed that the DNA methylation level at a specific CpG explained why a low-protein diet during development could induce a decrease in weight in adult mice that depends on the individual genotype. It was found that weaning weight in mice exposed to prenatal protein restriction was inversely related to the level of methylation of a specific CpG, 133 bp upstream of the transcription start site of the rDNA locus, but only for animals carrying the A allele at the genomic position 104 bp upstream of the TSS. Methylation of the CpG-133 was unaffected by environment for animals carrying the C allele. This epigenetic response to the environment, in interaction with genetics, is correlated with transcriptional and phenotypic results (Holland *et al.* 2016).

A notable example is also found in studies of psychiatric disorders related to childhood trauma (see Klengel and Binder 2015). An allele in intron 2 of the *FKBP5* gene - involved in the regulation of the glucocorticoid complex governing the stress response - has been shown to alter chromatin conformation and increase gene expression by bringing the promoter closer to an enhancer when associated to the glucocorticoid receptor (GR). This increase in *FKBP5* expression impairs the feedback effect that reduces blood cortisol levels inducing an altered physiological response to stress. Strikingly, the risk allele significantly increases the possibility of psychological disorders when the individual is subjected to trauma in early life, while it does not when the trauma occurs in adulthood. The underlying G×E mechanism seems to be epigenetic in nature: a severe stress in childhood in individuals carrying the risk allele determines a stronger stress-induced cortisol response that in turn induces an allele-specific demethylation at a GRE (Glucocorticoid Response Element) site in intron 7 of *FKBP5*. This demethylation is retained in adulthood and increases the risk of developing psychiatric disorders such as major depression and post-traumatic stress disorder. The same allele-specific epigenetic mechanism in interaction with the environment was also found in patients with psychosis in a recent study (Mihaljevic *et al.* 2021).

These examples illustrate the way by which the environment may act on the regulation of gene expression through epigenetic mechanisms: CpG methylation plays a major role in regulating gene expression, directly through modifying DNA conformation, and through governing the accessibility of transcription factors (TF). Depending on the TF, its ability to bind to its TF binding site (TFBS) is decreased (most often) or increased (notably for developmental TFs) according to the level of DNA methylation of the region (Yin *et al.* 2017). Therefore, a modification in the level of DNA methylation at specific sites triggered by the environment may change the expression of specific genes.

G×E genome-wide analyses

Few studies have analysed the epigenotype as a mediator of the G×E interaction on a genome-wide scale.

In human, Teh et al identified 1423 regions whose methylation levels were highly variables between individuals, by analyzing umbilical cord samples from 237 babies (Teh *et al.* 2014). While the methylation level of 25% of these regions were explained by the genotype, 75% were explained by the interaction between genotype and prenatal environment (maternal age, body mass index, smoking, depression, etc.). The environment alone, independently of the genotype, had no significant influence. These findings were recently confirmed in a larger study including 2365 newborns (Czamara *et al.* 2019). The association of genetic variability with prenatal environment is thus the best predictor of DNA methylation variability.

In fish, Lallias et al. used several rainbow trout isogenic lines, characterized by a lack of genetic variability within lines, to study genetic differences in the impact of environmental variation on DNA methylation (Lallias *et al.* 2020). By incubating eggs at two different temperatures (11°C and 16°C), they showed that the magnitude of the environment-induced modifications of DNA methylation profiles was dependent on the genetic background.

Structural characteristics of genomic regions showing G×E interactions

Current research suggests that environmentally-responsive epigenetic regions may show specific characteristics (Law and Holland 2019). Correlated regions of systemic interindividual variation have been observed, often associated with transposable elements and subtelomeric regions (Gunasekara *et al.* 2019). These regions are remarkably independent of the overall tissue specificity of DNA methylation levels, as when observed in one tissue, they are predictors of DNA methylation levels of other tissues in the same region. They are partly governed by genetic variation, and partly associated with the early life environment.

Genes showing G×E interactions seem to bear longer intergenic lengths, high motif concentration, and mid-range expression levels (Grishkevich *et al.* 2012). In addition, genes with G×E interactions are more often associated with distant-acting loci than genes without G×E interaction (Smith and Kruglyak 2008).

Conclusion

Although the chicken was the first species of agronomic importance to be sequenced, less experiments than in mammals have been conducted in birds to study epigenetic processes, despite their possible influence on economically important phenotypes. Given the likely evolution of the climate in the near future, there is a need to improve the adaptive capacities of animals to climatic and dietary changes. A better understanding of the epigenetic mechanisms governing the genome responses to changing environments could open up new avenues for improving selection for a large range of traits including animal welfare. Deciphering the contribution of epigenetic effects to G×E interactions is therefore a promising area of research, not only for the understanding of the molecular mechanisms involved, but also for its possible applications in livestock production. With the ongoing technological developments in genomics and epigenomics and the decreasing costs of sequencing, it is possible that future breeding strategies will not only use genetic information but also epigenetic analyses.

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Robotics and big data for precision farming

Robotics and big data for precision farming (T. Norton)

Thomas NORTON

Division Animal and Human Health Engineering, Group of M3-BIORES: Measure, Model & Manage Bio responses, Catholic University Leuven, Kasteelpark Arenberg 30, 3001 Heverlee, Belgium

tomas.norton@kuleuven.be

Introduction

Precision farming is an approach to the design and control of farming operations and has largely applied in the crop production sectors. It is considered to be part of farm management and specifically focuses on controlling inputs based on the intensive monitoring of the growing process. The goal is to maximise the spatial-temporal productivity of the crop given the constraints faced by modern farming and under the governance of a holistic economic model of the farm. In this way resource efficiency is optimised whereas the genetic potential can be better realised. In the field of livestock production precision farming research and development is being done within the concept of Precision Livestock Farming (PLF), which is being driven by similar demands seen in the crop production sectors but with the added complexity of upholding animal welfare as part of sustainable and productive animal production.

In recent years we have seen remarkable developments in technologies to monitor various animal productivity, health and welfare indicators on an individual and group level. Precision Livestock Farming (PLF) has emerged with the focus on linking these indicators with technologies such as sensors, data analysis and visualisation software and robotics in a way that empowers livestock farmers to sustainably manage their farming practices. In doing this there is no doubt that other stakeholders in the value chain can also be implicated in a positive way. For example, animal breeders, feed developers for example can use the information derived from these technologies to improve the inputs to the farming system so to help farmers balance target for efficiency, welfare and environmental impact.

Here, we present the core principles underpinning the innovation of sensing systems the monitoring large quantities of generated data for animal monitoring followed by the possible technologies that can address the needs of the poultry sector into the future. The emergence of robotic systems that have the potential to better support poultry farmers in a spatial-temporal manner are also presented.

Potential of precision livestock farming Tools in modern poultry production

The core principle of PLF systems is to continuously measure the responses of the animals directly rather than indirectly from the environment surrounding the animal. Enhancing the farmers' eyes, ears and perhaps nose by monitoring animals is only possible through fully automated continuous (7 days a week, 24 hours a day, 3600 seconds per hour) direct monitoring of animals. By accumulating these responses interesting information on the health welfare of the animal can be derived. Modern technologies such as climate control and financial programmes already measure a number of parameters on the farm. However, PLF technologies allow the animal to be the central concern.

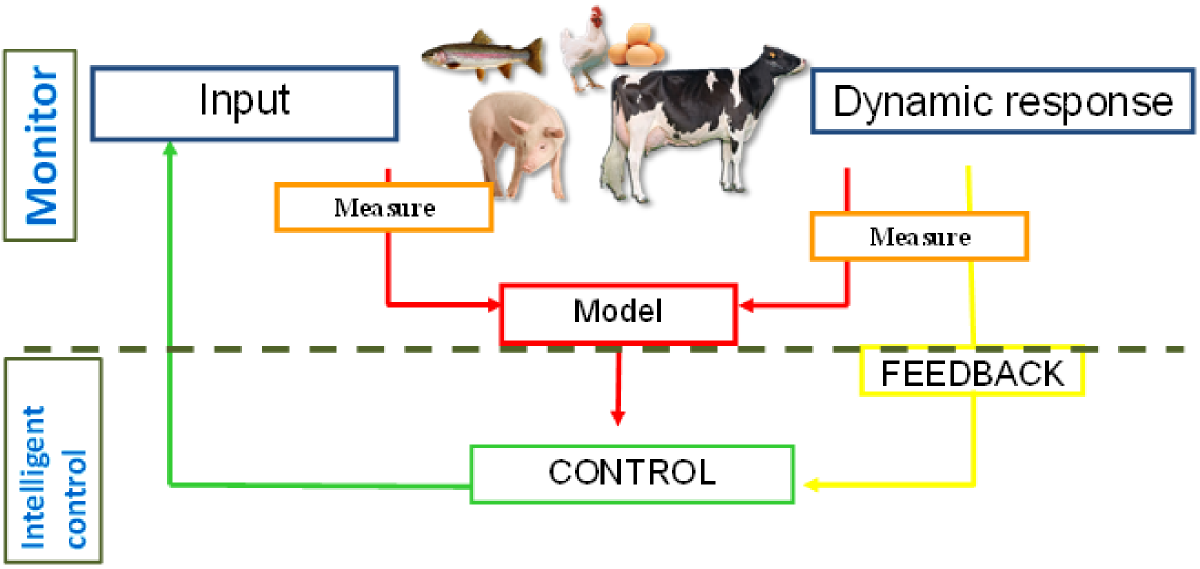

Figure 1. The basic scheme of Precision Livestock Farming (Berckmans, 2006)

A key criteria of measuring animal responses is to realise it in a cost effective, robust way. This is why sensors like cameras and microphones are extremely useful. There is no physical contact with the animal so there is no risk of influencing the animal. The cost to the farmer can be also be kept minimal, as many animals can be monitored using a small number of sensors. On the other hand wearable sensors have the some unique opportunity too being attached to the animal, so that no behaviours go unobserved. Moreover, while the application of sensors and accompanying ICT systems is a central part of Precision Livestock Farming (PLF), there is still further room for progress in robotics to remove the simple monotonous tasks from the hands of the farmer in the poultry industry. By doing so farmers can focus on other aspects of their business, as well as on improving standards of care for their animals. Moreover, through the uptake of robots, the role of the stock person can change; robots give the opportunity for their time to be used differently. This review will briefly cover PLF technologies including the potential for moveable robotics to monitor and manage poultry facilities.

Examples of data analysis and robotics for precision farming of poultry

Behaviour monitoring of Broilers and Layer Hens

Camera-based continuous monitoring of broiler flock behaviour has been demonstrated as a means of detecting daily problems poultry houses with large flocks (de Montis et al., 2013). For example, abnormal behaviour of broiler flocks can be detected using algorithms to measured occupation patterns of animals with a forecast of what this should be at different times during the day (Kashiha et al., 2013). Peña Fernández et al., (2018) showed in recent research using this activity and occupation-based monitoring approach has that correlations exist between activity and distributions indices and two important welfare challenges, namely footpad lesions and hock burns. There is a positive relation between occupation pattern deviations and scores of foot pad lesion. This indicates that birds that tend to group for longer periods face an increased risk of food pad lesion infection. The negative relation between activity pattern deviations and the hock burn scores indicates that increasing the activity of the flock would also reduce the risk of hock burn in the flock. This corresponds with previous finding that less active broiler chickens kept on badly conditioned litter can worsen this kind of lesion (Haslam et al., 2006). Further work has been done on using other image derived variables such as optical flow to track flock behaviour deviations (Dawkins et al., 2021). This approach derives features from the general movement flow patterns of birds, which have been shown to be correlated with health and welfare indicators.

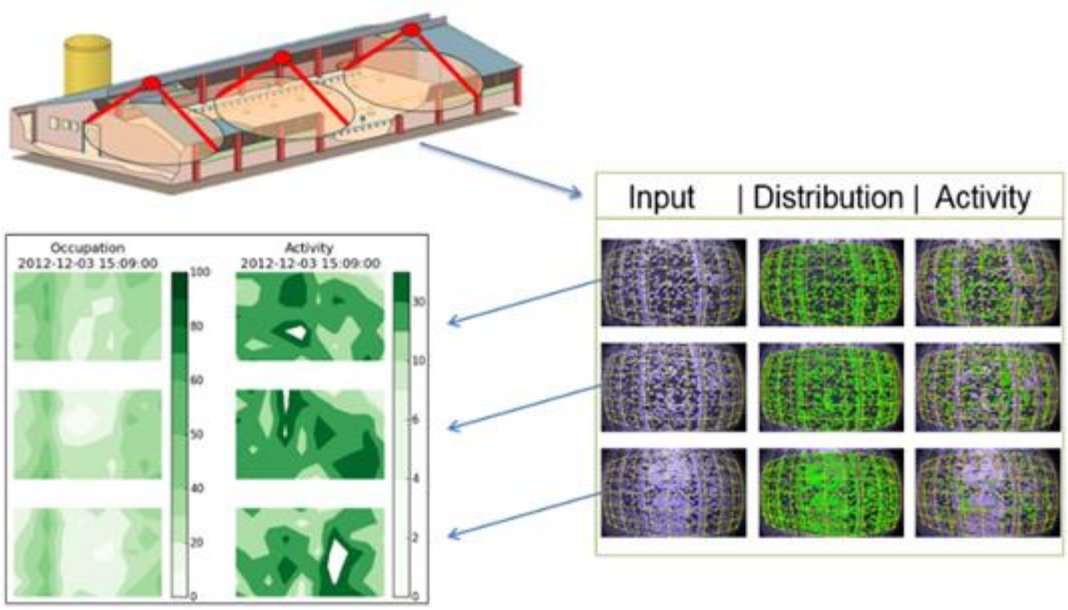

Figure 2. Two different application of the same camera-based solution for behaviour monitoring in poultry. On the left is a tool for sleep quality analysis of layer hens and on the welfare monitoring of broiler chickens.

The same camera technology can be applied for the monitoring of layer hen behaviour. Willems et al (2022) have recently developed a camera-based tool to aid in estimating the infestation level of the poultry red mite (PRM), Dermanyssus gallinae, in a layer house, as this is an important ectoparasite that severely affects layer hen health, welfare and productivity. This mite represents a major threat to the egg production and monitoring and management of PRM is very difficult in practice, as it tends to hide in crevices in poultry houses during the day, and then becomes active during dark periods to feed on the hens' blood. Because the PRM infest the bird plumage the hens become irritated and display restless behaviours during dark hours. Willems et al. (2022) have identified these behaviours and then capture the related change in behaviour through computer vision algorithms. Activity changes were captured using a Gaussian Mixture Modelling approach. As seen in Figure 2 a two-dimensional heatmap was created and, in turn, used to extract a feature reflecting the sleep quality of the birds. The outcomes can be used as a monitoring tool that indicates to PRM infestation levels.

Automated systems for monitoring and management of poultry flocks

Growth management of broilers

There has been much work done be geneticists in order to improve leg performance in broiler chickens. However, this work takes much time, and while genetics are improving there is still much work needed to actively manage the leg problem currently on broiler farms. One approach is to tightly manage the feeding of broilers so that the compensatory growth can be actively controlled birds so that birds increase weight their legs in accord with their physical development. Compared to ad lib feeding it might be interesting to actively control the growth trajectory (weight as function of time) of broilers in a more economical way to reduce mortality and leg problems. A key aspect of such as control processes is the growth trajectory model. Such as model can be built developed when accurate feed intake per bird and as well as weight of broilers are available. The dynamic relationship between the process input feed and the process output weight can be determined and used then to predict the dynamic weight response to feed supply. Based upon such prediction one can calculate how much feed is needed to get a certain weight response. The work of (Aerts et al., 2003) have demonstrated that a real-time model-based controller of this type is to be able to realise a desired trajectory of growth in broilers.

Activity stimulation of broilers

An approach to manage the high incidence of leg weakness in modern broiler chickens can also be done by encouraging more activity during the grow-out. Like all animals, chickens are influenced by their micro-climate one potential stimulator of activity of birds is to change their environment. This of course is not the only or best stimulation option. In their study, Youssef et al. (2015) developed a controller that is able to manipulate, spatially, the activity level of chicken within the ventilated chamber by paying attention to most constraints (e.g., limitation of the heating and ventilation systems) and the control objectives (e.g., avoiding heat/cool stress by limiting the control actions to be within the thermoneutral zones of the birds).

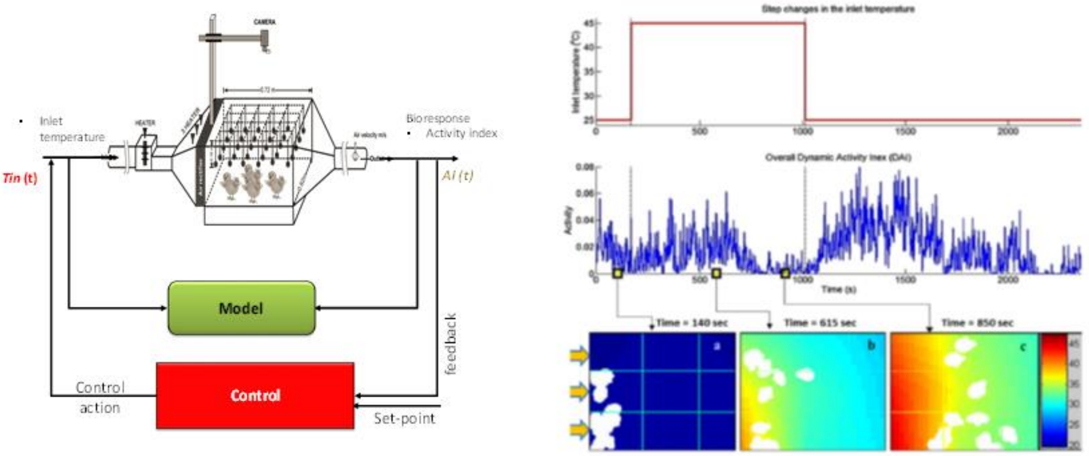

Figure 3. The climate control chamber for bird activity control and the control scheme applied during this research, (left) The activity response of chicks within the climate chamber under step input in temperature (right).

Figure 3 shows an example of the behavioural responses of the birds inside the chamber to a step increase in the inlet temperature from 25 ^{o}C (heater power is minimum) to 45 ^{o}C at constant ventilation rate of 4.5 m^3 h^{-1}. At low ambient temperature (25 ^{o}C) the birds occupied the zones closer to the heating source tended to get closer to each other (huddling) and showed low overall activity in the chamber (<0.02). During the transient phase, when the ambient temperature started to increase, the chickens started to show an increase in their overall activity index (AI) (>0.02) and move away from each other. At the steady-state the ambient air temperature in the zones close to the heating source (zones 1, 4 and 7) had reached a high level (>41 ^{o}C) causing the chickens to move away from these zones (with an average time constant of t ¼ 4.2 min) and spread away from each other. This research shows it can be possible to control the activity.

Moveable robots for automated management actions in poultry houses

Robotics is already present in the poultry production sector for many years. An example of a robotic process in the modern cage egg industry is the use of integrated conveyor belts to collect and transport eggs to the packing room. In recent years we have seen the introduction of autonomous vehicles that are capable of moving throughout the poultry house, monitoring the environment and also performing a number of tasks, which have the potential to save the time of the farmer.
Examples include:

Litter quality monitoring: Farmers must continuously monitor the litter quality in the house, as it is well known that moist poultry litter can compromise health and welfare. The opportunity for a robotic system is to sample litter quality sampling and litter conditioning when crusting or moisture saturation has occurred.

Abnormal behaviour monitoring: for broiler chickens an important welfare indicator that is currently measured via 'gait scoring' with human observation. The opportunity for robotics is to monitor bird movement monitoring system in order to quantify bird walking ability by assessing the interaction of the robot with the bird.

Dead bird detection: Every day the broiler farmers need to remove dead birds from their buildings – this is not an enjoyable task for any farmer. Robotic technologies are now being brought to the market with the application of dead bird detection in mind. Currently there are a small number of robotic solutions each providing a quite specific set of applications and each with their own unique value proposition. In the future, the prospect of modular robotic platforms, such as that conceptualised in Figure 4, which allow for different applications to be realised with the same unit. This could realise interesting opportunities to the poultry farmer in the future.

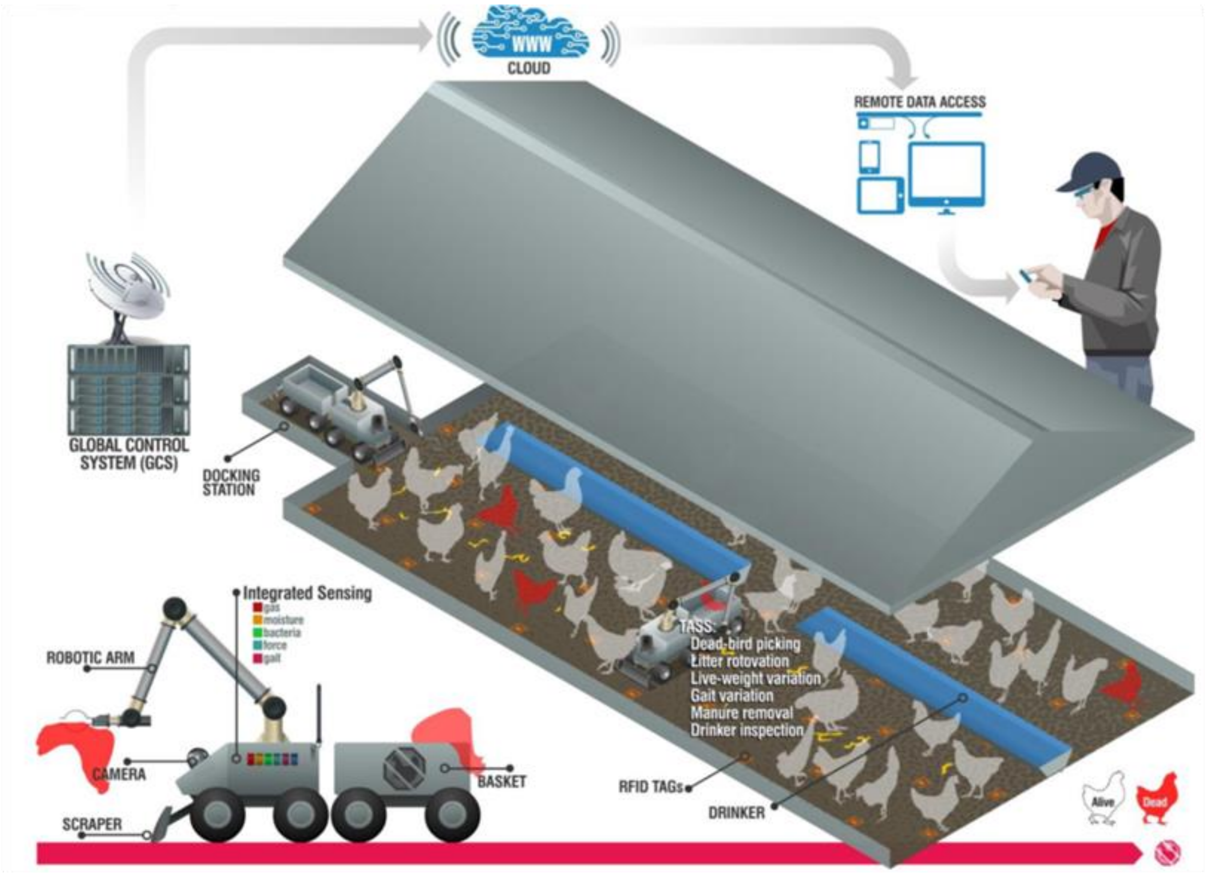

Figure 4. Concept for robot-support poultry farming of the future

Conclusions

Developing PLF from a concept towards viable opportunity for poultry farmers has already started. PLF systems are becoming available in products and are getting operational in commercial farms. The key challenge is not the innovation of technology but to discover how these technologies can create value for the animals and the farmers. The fundamental advantage is that PLF systems are monitoring continuously and 7 days a week and 24 hours a day. This is much more than what any farmer or human observer can expect to do without the help of technology. Other ways in which PLF can create value include: saving labour time, saving time in detecting problems, giving less stress to the farmer, solving problems on the spot immediately instead of later for other animals, giving social recognition to the farmer, giving quantitative numbers about what happens to the animals and others.

While it is clear that opportunities for sensor and data-driven applications exist multi-disciplinary and multi-sectoral collaboration must be realised to make these systems become successful support tools in the field. Most importantly, the needs of the farmer can only be fully represented if he is part of the innovation process.

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SPECIALIZED SESSIONS

Education

Poultry science education: Issues and perspectives (S. Yalcin)

Servet YALCIN

S. Yalcin[1], M. Cassandro[2], J. Roberts[3], E. N. Sossidou[4] [1] Ege University, Faculty of Agriculture, Department of Animal Science, Izmir, Turkey, [2] University of Padova, Department of Agronomy, Food, Natural Resources, Animals and Environment, Legnaro, Italy, 3 University of New England, School of Environmental and Rural Science, Australia [4] Veterinary Research Institute, Ellinikos Georgikos Organismos, DIMITRA, 57001, Thessaloniki, Greece

Corresponding author: servet.yalcin@ege.edu.tr

Abbreviated title: Education in poultry science

Summary

The poultry sector is one of the most intensive and efficient farming systems in the world. The projected global population of 9.5 billion by 2050 will increase the demand for food and animal-based protein, and it is called to do so in an environmentally sustainable way. Therefore, the growth of the global poultry sector is expected to continue. Consequently, poultry production will require large numbers of well-educated, well-trained poultry experts. Currently, poultry science programs are primarily offered in animal and veterinary sciences at universities at the undergraduate, graduate, and Ph.D. levels. Effective education programs require a multidisciplinary approach employing health and welfare, biology, genetics, management, nutrition, immunology, physiology, environment, biotechnology, biodiversity, economy, and social aspects. Moreover, as the poultry industry has a range of technological and sensor instruments to increase animal productivity, continued development and training of new technologies will be necessary to support the industry and precision poultry farming. On the other hand, consumers' food selection is influenced by many factors, including ethical standards through rearing and processing. Public concerns about poultry production systems have created alternative systems in areas such as free-range chicken meat and eggs, and organic meat and eggs. The media play an important role in consumers' willingness to eat poultry products; however, there is a need to establish an effective mechanism for the dissemination of knowledge.

Working Group 11 (WG11) of the European Federation of WPSA, entitled 'Education and Information' was established to support the needs mentioned above. The main objectives of WG11 are to encourage poultry education, stimulate universities and colleges to offer poultry courses at different levels of education, exchange scientific and technological information between universities and industry, in permanent learning, and disseminate information to increase public understanding of chicken production and products. In this respect, WG11 members have been regularly involved in WPSA Congress and other WPSA-sponsored conferences to provide opportunities for youth to learn about and experience careers.

Keywords: poultry science, education, university, training programs, poultry industry

Introduction

When poultry production is mentioned most people have an image of raising backyard chickens. However, science, technology, and highly mechanized operations are involved in modern poultry production. Today, poultry production constitutes the main part of the livestock sector in the world. Global poultry meat production reached 28 million tons and egg production was around 80 million tons in 2019. The estimated chicken population was 23.7 billion in 2018 (Statistica, 47 2020). Although demand for egg and chicken meat is increasing worldwide, poultry producers are faced with many challenges, including changes in consumer preferences, trust in product quality and safety, better animal welfare conditions, disease, climate change, and environmental pollution.

Considering the sector's contribution to human nutrition, food security, and the economy, poultry education is key to increasing our knowledge and expertise. Poultry education may take forms at different levels, including students, technicians, researchers, farmers, and consumers.

This paper focuses on the need for poultry education, extension, research, and development.

Students, technicians, researchers

To supply egg and chicken meat to the world's population, poultry production systems must be efficient and sustainable. Thus, a comprehensive understanding of poultry production systems is necessary. A poultry science education offered by several faculties such as Agriculture Faculties, Veterinary Faculties, Life Sciences, Environment, Food, and Natural Science gives opportunities to pursue a career in a wide range of fields. In Europe, poultry science departments are in animal science disciplines while there are 6 universities that have a separate poultry science department in the US.

Contrary to popular belief, a poultry science degree does not just mean gaining practical experience in rearing and feeding the chicks. It means gaining the ability to research and analyze, cooperate and work with people, as well as critical thinking to solve everyday problems. The poultry sector has undergone major structural changes, become multi-nationals during the past two decades. Thus, a poultry science curriculum must be internationalized by enhancing international cooperation between universities through faculty and student exchange (Leenstra et al., 2010), productive and interesting for the students and must include the needs of a changing poultry industry (Sossidou and Niebuhr, 2013). The curriculum must contain subjects associated with breeding, management, nutrition and feeding, reproduction, poultry health and welfare, and processing of the products. At the same time, students should be encouraged for a complete understanding to explore support subjects such as biochemistry, genetics, food science, business, and economics. The program also should offer an internship program for students to learn practice working at a farm. Internship programs include a variety of areas such as broiler and egg-laying management, hatchery management, feed mill, and processing management. Throughout the internship, students also learn teamwork.

Online and distance poultry education are also available (Sossidou et al., 2008). Early distance education programs were provided only through the mail. However, technology has led to high quality educational opportunities made available via online learning. Today, a non-thesis MSc degree program is offered by universities (University of Edinburgh & SRUC, Texas A&M) to provide a flexible learning environment for obtaining the necessary skills to address sustainable poultry production. In the spring of 2020, many universities provided free access to online courses for undergraduate and graduate students to minimize the impact of the coronavirus outbreak on students and to keep the campus community safe. Course materials such as videos of lectures, slide presentations, and other resource materials can be viewed by students.
There are also online courses for individuals who are seeking to increase knowledge about poultry science in the area of breeder & hatchery, waste management, and meat production (Texas A&M). Unfortunately, the master's program in avian health offered by the University of Melbourne in Australia has recently been discontinued,

Furthermore, post-graduate courses on specific topics are organized commercially such as by the World Veterinary Education in Production Animal Health, Aeres Training Centre International, Wageningen Academy (The Netherlands), Texas A&M (USA). WPSA and Poultry ScienceAssociation (PSA) symposia and seminars also can be considered as postgraduate training (Hunton et al., 2006).

Using the Scopus database, there is a census of 9,899 documents with Poultry Science words on the title or abstract or keywords, starting from 1911 (one document in 1911, with a maximum number of documents in 2013 with 911) to March 2022. Until 2000, the total documents on poultry science research were equal to 1,239, with an average number of documents per year of 13.9, after 2000 the total documents published in peer review journals increased dramatically to 8,660 documents with an average of 433 documents/year. The most important subject areas were: Agricultural and Biological sciences with 12,737 documents, Biochemistry, Genetics and Molecular Biology with 990, Veterinary with 904, Immunology and Microbiology with 760, and Environmental Science with 717 documents in total. These documents were published in the most popular scientific journals that are ranked as following in terms of total published documents: Poultry Science (3,564), British Poultry Science (1,087), Journal of Applied Poultry Research (1,048), World Poultry Science Journal (653) and Journal of Poultry Science (481).

The 10 most relevant Departments/Research Centers, in terms of total documents published on poultry science, are USDA Agricultural Research Service (507), United States Department of Agriculture (513), University of Arkansas (386), University of Georgia (270), Wageningen University and Research Center (249), North Caroline State University (236), Mississippi State University (219) College of Agricultural and Environmental Science Athens Campus (209), China Agricultural University (191), and Purdue University (185). These 10 Departments/Research Centers published 29% of total documents, from 1911 to March 2022. In terms of countries, the 20 most prolific countries, in research of Poultry Science, were: the USA (3362), China (8982), United Kingdom (753), Japan (606), Canada (551), France (338), Netherlands (332), Germany (306), India (295), Brazil (291), Iran (257), Australia (252), Spain (249), South Korea (248), Belgium (239), Turkey (231), Italy (189), Poland (146), Pakistan (144) and Israel (139).

Poultry farmers

There is a positive relationship between education and agricultural productivity (Xayavong et al., 2016; Gondwe et al., 2017), especially in developing low-income countries. Poultry farmers also need the information to improve their productivity. The most common information source for poultry farmers is the internet, friends, and extension services. Transformation of scientific results for farmers to use in practice involves tailoring the message. Bringing farmers and extension services together may create a collaborative climate to find solutions to industry problems. Extension programs are offered by departments of agriculture or industry representative bodies in a range of countries.

Moreover, in most regions of the world, small flocks of village chickens are in the hands of women. In addition to taking care of them, by providing additional feed and water, women often have control over the money resulting from their sales. It is important to acknowledge the different roles, interests, and needs of men and women. Several aspects need to be taken into consideration. Men and women or different groups in the community can have different interests (e.g. semi-commercial farmers versus village farmers). In the household, as well, interests might be different between different members according to their sex and their age.

Socioeconomic reasons, socio-cultural attitudes, and group and class-based obligations, influence men and women's roles, responsibilities, and decision-making functions. Cultural beliefs and practices limit women's mobility, social contact, access to resources, and the types of activities they can pursue. Institutional arrangements can also create and reinforce gender-based constraints or conversely, foster an environment in which gender disparities can be reduced. All these aspects, related to poultry production and culture to promote it, can affect women's and men's adoption of new measures, their possibility to scale up their production, or their participation in decision-making and social life in several environments and world regions (Bagnol, 2012).

Consumers

Poultry production provides chicken meat and egg at relatively low prices. Most people are concerned about how chickens are raised. Public concerns about industrial vertically integrated poultry production have created an interest in alternative production systems such as organic egg and chicken meat, free-range egg and chicken meat, and locally produced eggs and chicken meat. To promote sustainable economic growth, especially in developing countries, agro-tourism has also aimed to raise free-range chickens to provide egg and chicken meat to tourists (We et al., 2015).

Consumer decision to buy the product has a series of steps, including identifying the need, information, and evaluating alternatives, leading to the purchase decision (Burrow, 2009; Kasapidou et al., 2015). Usually, the public's knowledge of the poultry industry is limited (Erian and Phillips, 2017), with the media and the internet being the main sources of information. However, media notifications are not always correct. For example, media reports on egg cholesterol affected consumers' egg consumption behavior in the past. Similarly, the media have attempted to alert the public on the injection of hormones into chickens, genetic modifications of chickens, and ethical standards and practices through breeding, growing and slaughtering. Such misinformation passed on through media and social media may lead the consumer to not trust poultry products. Consumers should receive correct information and efforts should be increased to provide a consistent message concerning poultry production systems.

WG11 of Federation of WPSA

WG 11 of the European Federation of the WPSA aims to encourage collaboration in poultry education and exchange of scientific and technological information and stimulate cooperation between universities, researchers to exchange students, and information in the field of poultry production and management (Sossidou et al., 2014). The Mediterranean Poultry Network (MPN) has been functioning under WG11 since August 2009. The idea of the Mediterranean Poultry Network (MPN) of the World's Poultry Science Association (WPSA) was proposed by Prof. Ruveyde Akbay from Turkey, during the European Poultry Conference that was held in Verona in 2006 (EPC2006). The MPN aims to promote WPSA activities in all countries facing the Mediterranean Sea (Yalcin and Sossidou, 2010). In order to achieve this objective, six Mediterranean Summits were organized by Greece, Turkey, Egypt, Lebanon, Italian-Lebanon, and Italian branches of the WPSA. These summits aim to exchange scientific knowledge on the poultry sector and to create a "scientific osmotic flow" between private and public researchers involved in the improvement of the poultry sectors around the Mediterranean Sea and the world. The next summit will be organized by the Spanish branch of the WPSA at Cordoba, Spain, in June 2022.

Conclusion

Education, training, and extension for the benefit of the poultry industry are conducted in various ways in different countries around the world. The responsibility for these activities is borne by a range of entities, including universities, vocational training colleges, poultry companies, and industry representative bodies while the extent of these activities is frequently limited by financial resources and the availability of teachers and trainers. Cooperation among countries can be coordinated by W11 of the WPSA in order to make the most efficient use of available resources.

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Marketing and economy

Economical consequences of welfare on poultry production in France and Europe (A. Le Lay)

Aymeric LE LAY

A. Le Lay, S. Fourdin, L. Warin, P. Créach. ITAVI 7 rue du Faubourg Poissonnière 75009 PARIS, France

Corresponding author: lelay@itavi.asso.fr

Abstract

The historical segmentation of the French broiler market, from farm to fork, includes protected geographical indication (PGI), protected designation of origin (PDO), organic, Label Rouge (largely with IGP), the Certified (Certification of conformity, CCP) and the production standard (conventional). Animal welfare (AW) is a dimension of related products. However, new private AW-focused schemes have emerged since 2015. These schemes combine commercial claims with husbandry, transport and slaughterhouse requirements. These practices can have a negative impact on the cost of production in the various links in the chain, while adding value to the final product. The objective of this study was to characterize the emergence of AW-schemes and their impacts on broiler market segmentation. The focus is on France but is supplemented by a comparison with European neighbors.

Multi- and transgenerational effects of feeding strategies in poultry (J. Buyse)

Johan BUYSE

J. Buyse (1), J. Lesuisse (1), C. Li (2), S. Schallier (1) and N. Everaert (3) 3 (1) Laboratory of Livestock Physiology, Department of Biosystems, KU. Leuven. Belgium (2) New York University, Shangai (3) Animal Science Unit, University of Liege, Belgium

Abbreviated title: feeding strategies in poultry

Corresponding author: johan.buyse@kuleuven.be

Summary

Since the last two decades, a lot of attempts have been made to find an appropriate solution for the so-called broiler breeder paradox. This paradox states that is rather impossible to combine a high production/fast growth with an acceptable reproduction capacity and good health/welfare status in a body weight-controlled breeder hen.

The majority of these trials were aimed at the breeder generation, and can be classified as quantitative or qualitative management strategies. With respect to daily/weekly feed allocation programs, some benefits can be seen in a '5 days on and 2 non-consecutive days off' program and daily split feeding. In contrast, little gain is to be expected from qualitative dietary interventions (e.g. diet dilution), except maybe from reduced balanced protein diets. More recent attempts are now focusing on the carry-over effects of the imposed treatment on the breeder generation on performance of F1 offspring (multigenerational studies). Results so far are promising. This review paper closes with a report on an unique transgenerational study over three generations with broiler breeders and their offspring, focusing on the effects of a reduced dietary crude protein content for F0 and F1 breeders. This study identified pure transgenerational effects at the level of offspring performance, feather condition, polydipsia and some behavioral characteristics.

Key-words: Broiler breeders – feeding strategies - paradox – welfare – transgenerational effects – epigenetics

1. Introduction

Poultry meat production (mainly broiler meat) has increased tremendously during the last decades (Zuidhof et al., 2014). This increase is mostly due to an on-going selection for growth rate, breast meat yield and feed efficiency by yet a limited number of breeding companies. Moreover, not only the breast muscle and total meat output has increased, but also the time to reach the required slaughter weight has concomitantly remarkably decreased as well.

However, as a consequence, growth rate of the breeder stocks has increased as well. The pure line and (great) grandparent and parent breeders must carry the rapid growth and feed efficiency traits and pass it on to their (broiler) offspring. When breeders are fed *ad libitum*, they become obese and are prone to develop metabolic diseases, as humans do, and such flocks experience high morbidity and mortality. Clearly, such practice has a detrimental influence on the breeders' health and welfare (Decuypere et al., 2010, De Jong and Guémené, 2011). In addition, reproductive performance (e.g. egg production rate, fertility and hatchability) of these overweight hens is poor (e.g. Hocking, 1993; Taherkhani et al., 2010; Mohiti-Asli et al., 2012). In daily practice, a certain degree of controlled feeding (especially during the rearing period) is imposed as this management strategy improves the condition and health status of the breeders and their laying performance considerably. However, new welfare-related problems have arisen as feed-controlled breeders may have a hunger feeling and may display abnormal behavior (e.g. frustration, boredom, object and bird pecking). Hence when targeting the required growth pattern, it is apparently impossible to combine a high production/fast growth with an acceptable reproduction capacity and good health/welfare status and this concept is known as the 'broiler breeder paradox' (Decuypere et al.,2010). This paper aims to review the available literature in order to seek for or to propose acceptable solutions to solve – at least to some extent – this paradox.

2. Managing the breeder generation

Several attempts have been made to improve the welfare status related to the controlled feed allocation. These attempts can be categorized as quantitative (amount of daily feed allocation and feeding frequency, the timing and duration of the feed restriction, scattered feeding, etc.) or as qualitative (fiber addition, low-energy levels, supplements, etc.) strategies.

2.1. Quantitative feeding strategies

Morissey et al. (2014) compared a daily feeding practice with skip-a-day (SAD) feeding (both feeding frequencies combined or not with supplementation of fiber and the appetite suppressant Ca propionate) during rearing of breeder hens on performance and video-taped behavior during the feeding bouts. The authors concluded that there were no clear differences between the feeding frequency strategies with respect to suppressing the breeders' hunger feelings. Aranibar and coworkers (2020) provided breeder pullets with oat hulls on the off-day of SAD program, and observed better flock uniformity and higher egg production compared to a standard SAD program. The oat hull-supplemented breeders had occasionally higher plasma corticosterone levels at 48h after feeding. These hens had increased feeding behavior at the off-day (leading to satiety?) and hence a more similar behavior patterns on the on- and off-feeding days. Besides daily feeding and SAD programs, other strategies such as 4/3R (4 days feeding and 3 days of feed deprivation) and 5/2R (5 days feeding and 2 days of feed deprivation) were recently studied by Carneiro and coworkers (2019). The authors observed that hens reared according to the two latter programs (especially 4/3R) had better performance in terms of feed efficiency and egg numbers, fertility and hatchability (both tendencies) compared to the more traditional daily feeding and SAD programs. Unfortunately, no welfare-related parameters were studied. A feed deprivation period of more than 24h is not allowed anymore in many European and other countries (Morissey et al., 2014). Consequently, the practical relevance of 5/2R and surely 4/3R programs can be notwithstanding the fact that some studies (Arrazola et al., 2020) revealed that a 4/3R program decreased feed motivation and stress compared to a traditional daily feeding protocol. A viable alternative program could be found in the proposal of Lindholm et al. (2018) in which breeder pullets receive a daily amount on 5 days and are feed deprived during 2 non-consecutive days per week. However, the impact on bird welfare needs to be investigated further. Another promising feeding technique for the breeders could be found in split-feeding in which the daily allocated amount of feed is not provided in one single meal but distributed over two or even three meals. Indeed, amongst others, Taherkhani et al (2010) have shown that split feeding of 2 meals a day from 27 wk to 39 wk, improved cumulative egg production compared to the traditional single daily feeding. The impact of split feeding on welfare-related parameters should be investigated in more detail.

2.2. Qualitative feeding strategies

De Jong et al. (2005) postulated that low-energy diets for restricted breeders could improve their welfare as the birds would spent more time on feeding. In addition, more gut fill should induce satiety and hence reduces undesirable hunger feelings and associated behavior. During the first part of the rearing period, a low-density diet of 8.4 MJ/kg (compared to a standard 10.9 MJ/kg diet) indeed extended feeding time and reduced frustration behavior (reduced stereotypic object pecking). However, providing a very low-energy diet (9.2 MJ/kg) during the laying period led to increased stress as indicated by elevated heterophil to lymphocyte ratios. Mohiti-Asli and coworkers (2012) concluded that inclusion of 3 % fiber (cellulose more efficient than inulin) improved the reproductive performance of older breeder hens but no welfare-related parameters were measured. Cellulose (marginally although significantly) reduced feed intake of *ad libitum* fed breeders with 2g/d, suggesting an enhanced satiety/decreased hunger feeling. Feed dilution by adding fibre-rich ingredients such as oat hulls, sugar beet and sunflower (e.g. Decuypere et al., 2006) might look promising as time spend eating was increased whereas the prevalence of spot pecking and damaging pecking in feed-restricted breeders was decreased.

However, not all comparable studies reported beneficial effects on welfare with such practices. Indeed, one of the conclusions from the European Broiler breeder paradox project (Decuypere et al., 2006, 2010) was also that the welfare benefits of feeding diets containing high concentrations of fiber *per se* are negligible and that the major role of fiber is to dilute the nutritional value of the ration and hence to augment the daily amount of feed provided to the breeders.

Another dietary factor that might be of interest is the protein content of the breeder ration during the rearing as well as during the laying period. Van Emous et al. (2013, 2014) conducted an experiment with Ross 308 breeders to evaluate the effects of two growth patterns [high growth pattern (2,400 g BW at 20 wk) and standard growth pattern (2,200 g BW at 22 wk)] and two reduced dietary protein levels [reduction in amino acids of 8% (low protein diet) and 16 % (medium protein diet) compared to the isocaloric high protein diet] on breeder performance and on eating time (time spend feeding and resting), eating rate and some behavioural traits. It is worthy to note that the reduced protein diets were only presented during the rearing period from 2 weeks up to 22 weeks of age, followed by a normal breeder layer diet afterwards. It was hypothesized that a higher body weight and a reduced dietary protein content during the rearing period would increase eating time and so reduce stereotypic behaviour, hence leading to ameliorated welfare. Breeders pullets reared on the low and medium protein diet devoted 22% and 63% more time to eating compared to their high protein counterparts, whereas eating rate was reduced with 9% and 26 %, respectively. It was concluded that low dietary protein levels indeed affected positively some beneficial behavioural traits (longer time eating, less stereotypic object and animal pecking), more than the growth pattern. However, low protein diets are known to adversely impact feather condition, as feathers have a high protein content (Van Emous et al. 2014). The practical application of reducing the protein content during the rearing period looks promising, but more research is needed to investigate possible carry-over effects not only on performance but surely on welfare-related issues during the subsequent laying period.

Next to the qualitative and quantitative feed restriction strategies, autonomous feed restriction programs (e.g. dietary supplementation of appetite suppressors such as Ca propionate, low palatability diets), were investigated as well (Decuypere et al., 1994; Tolkamp et al., 2005) although not conclusive and inapplicable in practice because standard growth patterns are difficult to achieve. Therefore, there is still an urgent need to develop other (feeding) strategies to counteract the health and welfare issues common to the current feed restriction practices.

3. Multigenerational feeding strategies

So far, the effects of the feeding programs (quantitative and qualitative restriction) on the performance and welfare indices of the breeders themselves were discussed. A logical question would be if the dietary treatments imposed on the parent stock would also affect the performance, behaviour and physiology of the offspring.

Offspring of broiler breeder hens fed on low-density diets (-21% reduction in nutrient density) during the laying period (25-60 wks) showed a better embryonic development as a result of increased egg weight due to higher amount of higher white:yolk ratio (Enting et al., 2007a). In another study of the same research group (Enting et al., 2007b), breeders were fed on low-density diets (varying between a 11% and 23% reduction in nutrient density) both during the rearing and layer period. The results of this study revealed that feeding low nutrient diets to breeders can have positive effects on day-old chick weight, postnatal growth rate and humoral immune status, yet strongly depending on breeder age and egg weight. Besides nutrient density, it is worthwhile to investigate the effects of maternal dietary protein content on offspring performance. The study of Rao et al. (2009) revealed that a maternal low protein diet for breeders can program the offspring as reflected in a lower hatch weight but higher post-hatch growth up to 4 weeks of age. In addition, changes in a whole array of physiological parameters such as plasma hormone levels and expression of some genes in the yolk sac membrane, muscle and hypothalamus of the offspring of protein-restricted breeders were measured. (Rao et al., 2009).

In continuation of their experiment on the effects of growth pattern and dietary protein content of breeders during rearing on breeder performance (Van Emous et al., 2013, 2014), embryonic development and performance of F1 offspring broilers were studied as well (Van Emous et al., 2015). Clearly, rearing the breeder pullets to a higher adult body weight at 20 wk resulted in significant positive effects on embryonic parameters (better fertility and hatchability of set eggs and reduced early embryonic mortality) of eggs collected from 29 wk-old breeders as well as a strong tendency towards better offspring performance. The dietary protein content of the rearing diet had apparently only few effects on these parameters. In a follow-up study, Van Emous et al. (2018) investigated the effects of a low protein diet (reduction of 15 g CP/kg) and early (21 wk) or late (23 wk) age at photostimulation on egg production, hatchability and offspring performance. There was a tendency that older (>46 wk) hens reared on a reduced protein diet produced somewhat less eggs and with a reduced hatchability. Advancing the age at photostimulation led to an overall increase in number of chicks per hen and improved cumulative feed efficiency of the offspring. In agreement with their previous study (Van Emous et al., 2013, 2014), a reduction in the protein content of the rearing diet did not affect progeny performance.

In our opinion, multigenerational studies refer to studies in which the parent population (F0) is treated in some way and how this treatment alters the performance and physiology of the F1 offspring. Manipulation of the incubating avian egg and looking at its effects on performance and physiology of the embryo and postnatal chick can be considered as a special case of multigenerational transmission. As an example, partial removal of albumen (3 ml) of layer strain eggs before the initiation of incubation impaired growth rate of pullets, egg production (both number and weight of eggs), and induced changes in carbohydrate and protein metabolism and differential gene expression and DNA methylation pattern (Willems et al., 2016). In addition, F1 offspring of parent hens that hatched from eggs with reduced albumen content displayed a temporary reduction in growth rate as well as an increased sensitivity to insulin (Willems et al., 2015). Hence, by using an avian model, prenatal protein undernutrition induces long-term alterations in production characteristics of the parent generation and multigenerational effects on F1 offspring.

4. Transgenerational studies

It can be inferred that there is already a lot of information available regarding multigenerational effects in mammals and some avian species. However, in contrast to these multigenerational studies, little is known about transgenerational (F0 up to the F3 generation) dietary manipulations in broiler breeders.

We have therefore performed a transgenerational study over three generations with broiler breeders and their offspring, focusing on the effects of a reduced dietary crude protein (CP) content (-25 % CP) in the diet of the breeders (F0 and F1 generations) during both the rearing and laying period.

In brief, the F0 generation of pure line A breeder hens (n=160) were provided with either a standard diet (control C diet) or a reduced balanced protein (RP) diet. Compared to the C diet, the isocaloric, RP diet was formulated to have a 25% reduction in crude protein and also in amino acids (for details on diet formulations and breeder management, see Lesuisse et al., 2017; Lesuisse et al., 2018a). The RP hens received on average 10% more feed than the C hens in order to achieve a similar growth trajectory. Hens were artificially inseminated and eggs were incubated under standard conditions. Day-old chicks were reserved for establishing the next F1 breeder generation or were raised as broilers (F0 broiler offspring). For the F1 generation, female chicks of both F0 groups were further subdivided in a C and RP group, resulting in four F1 breeder groups: C/C, C/RP, RP/C and RP/RP (the first and second letter refer to type of feed in the F0 and F1 generation, respectively). Next, an F2 breeder generation was created, which all received the C diet: C/C/C, C/RP/C, RP/C/C, RP/RP/C (the third letter refers to the type of diet for the F2 breeders). Within each generation, several broiler offspring trials were conducted with sexed broilers reared on isocaloric diets with varying protein levels. It needs to be addressed that pure transgenerational effects can only be observed in the broiler offspring of the F2 breeder hens as the germ cells for generating the F2 breeder generation are already present in the F0 generation. For the F0 and F1 generation, RP breeders were characterised by a higher body fat content, poorer laying performance and lower egg weights compared to C breeders (Lesuisse et al., 2017, 2018a). On the other hand, F2 hens from the RP/RP/C group realized the best laying performance, yet egg and day-old chick weights were lower compared to that of C/C/C hens. F0 and F1 broilers originating from RP breeders realized a higher slaughter weight and/or improved FCR, which can be linked to their better total tract nitrogen retention (Lesuisse et al., 2018b). When fed a standard broiler diet, male broilers originating from the C/RP/C breeders had a lower body weight than the broilers coming from C/C/C and RP/C/C hens. Thus, feeding the RP diet in the F1 generation reduced growth rate of the broiler offspring. However, when the F2 offspring broilers were reared on a low protein diet (15% reduction in crude protein), the C/RP/C broilers had a higher BW compared to their C/C/C and RP/RP/C counterparts. On the low protein diet, feed efficiency and body composition of F2 offspring broilers varied according to the nutritional history of their (grand) parents, suggesting pure transgenerational effects. Besides studies on breeder and their broiler offspring performance, welfare-related parameters were investigated as well (for details see Li et al., 2018, 2019a, b). Clearly, litter moisture content of pens housing C breeders was much higher compared to that of RP breeder pens, resulting in an associated increase in foot pad dermatitis incidence. In agreement with the observations of Van Emous et al. (2013), providing the RP diet to breeder hens (both F0 and F1 generation) resulted in a poorer feather condition. Surprisingly, the RP/RP/C hens had the best feather condition compared to the other three groups. The nutritional status of breeder hens did not have an impact on learning ability or memory retention but RP hens displayed more frustration-related and ‘craving for protein’ behavior. F2 hens descending from RP/C or RP/RP hens however were characterized a reduced frustration behavior and polydipsia. In conclusion, transgenerational effects of the maternal dietary protein content on feather condition, polydipsia and frustration-related behavior were undoubtedly observed.

The results clearly indicate that it is possible to program the offspring performance by altering the diet of the parent and even the grandparent breeders. The induction and/or inheritance of some effects without selection or altering the DNA sequence of the hens imply that epigenetic mechanisms must be involved. Hence, alterations in gene expression and possible causal DNA methylations and histone modifications being investigated in order to explain the observed results. Feeding these RP diets to breeders showed clearly positive effects on a broiler level by reducing FCR, increasing daily gain and improving the nitrogen retention, however breeder performance was negatively influenced by the RP diet, leaving also options to improve breeder performance by optimizing nutritional formulation for these RP diets. The liver transcriptome of the F1 generation at 44 weeks of age showed that the RP diet downregulated the amino acid metabolism. In addition, the RP diet activated the fatty acid metabolism for F1 hens descending from control fed F0 hens, while a dietary effect was observed on the glucose metabolism for hens descending from RP fed F0 hens.

5. Conclusions

It is appreciated that many attempts have been made to solve the so-called broiler breeder paradox. There seems to be little scope for doing this by focusing solely on the management of the breeder generation. In contrast, it is believed that benefits can be found in a multigenerational and definitely a transgenerational approach. More in particular, a balanced reduction in the protein content of the breeder diet elicits pure transgenerational effects at the level of the offspring, while also ameliorating the welfare status of the breeder generation. Attention should however be paid to the reproductive capacity of the breeders but this is pure a matter of optimizing the breeder diets.

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Turkeys

Some current factors and problems that influence turkey production and health (M. Hafez)

Mohammed HAFEZ

Former Head of Institute of Poultry Diseases, Faculty of Veterinary Medicine, Free University Berlin, Germany

Summary

Currently several factors and problems influence the turkey production worldwide. These include strong global competition, continuous changes of consumer perceptions with regard to food safety, animal welfare and environmental protection.

The loss of consumer confidence and trust in the quality and safety of poultry meat and poultry products will remain a major challenge. Several human foodborne bacterial infections are linked to poultry and poultry products. Control and/or elimination of these organisms present a great challenge. The development of antibiotic resistant bacteria will also be a continuous public health hazard. The future concept of turkey health will cover not only the absence of disease in birds, but also the relationship between the health of animals and their welfare. It will also take into account social, economic and ethical considerations, as well as support the achievement of a high level of environmental protection.

Emergence and re-emergence of infectious turkey diseases will remain an important non-ending challenge. Currently, only a few authorised pharmaceutical veterinary products will be available for the treatment of turkeys as food producing animals. The development of efficient vaccines against bacterial infections will lead to a reduction of the use of antibiotics and subsequently will reduce the development of resistant bacteria. Genetic resistance and selective breeding to improve production traits and health is a long-standing goal of the turkey industry. Improvement of rearing technology, management and nutrition will help to maintain bird comfort.

Finally, farmers, veterinarians, stockholders and all other partners involved in the production chain need to share more responsibilities.

Introduction

The modern turkey industry aims to high production and better quality at a low cost as well as constant, efficient and goal-oriented healthcare to prevent the development of diseases. Currently and in future several challenges and problems, will face everybody involved in the turkey production chain. These will include, strong global competition; changes in social, political and consumer perceptions related to food safety; animal welfare; environmental protection; a steady increase in the cost of feed; the emergence of new and unforeseen diseases, and new legislations. The present paper describes the main challenges facing the turkey productions. costs. In the future, the feed industry will be forced to take more responsibility for the feed ingredients quality and ensure that no avian pathogens and unwanted contaminants or residues are present in the feed.

Turkey diseases will remain the major challenge. Once an outbreak of a given disease occurs, it can explode into an epidemic and significantly negatively affect trade in a specific country, a continent or even globally. Also, climatic changes and limited water resources should be considered to influence production costs.

Strong global competition between producing countries

Competition and varying production costs in different countries will lead to increases the risk of introducing diseases to areas that are now considered to be free from such diseases. The steady increase in the cost of feed will accelerate the global trade. In addition, the increase of biofuel and biogas production will reduce the available land for food grains and feed production, leading to a considerable increase of feed costs for animal production. In the future, the feed industry, however, will also be forced to take more responsibility not only for the quality of the feed ingredients, but also to ensure that no avian pathogens and unwanted contaminants or residues are present in the feed. Furthermore, climatic changes and limited water resources also need to be seriously considered, as they will have an influence on the cost of production (Hafez, 2009). The top 10 producing countries are listed in the table 1.

Table 1: ***Ranking the top 10 World's turkey meat-Producing countries***

Rank	Countries	Production of Turkey Meat 2019 (Tons)
1	USA	2,638,803
2	Brazil	613,332
3	Germany	471,000
4	Poland	385,560
5	France	317,000
6	Italy	300,700
7	Spain	212,790
8	Canada	165,170
9	United Kingdom	148,000
10	Israel	94,429

https://scienceagri.com/10-worlds-biggest-turkey-meat-producing-countries/

Changes in consumer perceptions

Food safety

There is a higher interest from the consumer in organic poultry production and a great demand for natural and healthier products. The loss of consumer trust and confidence in poultry meat's quality and safety is a significant challenge. Turkey meat can harbour different foodborne pathogens such as Salmonella serovars and Campylobacter spp., which are the most common causes of human foodborne infections linked to poultry.

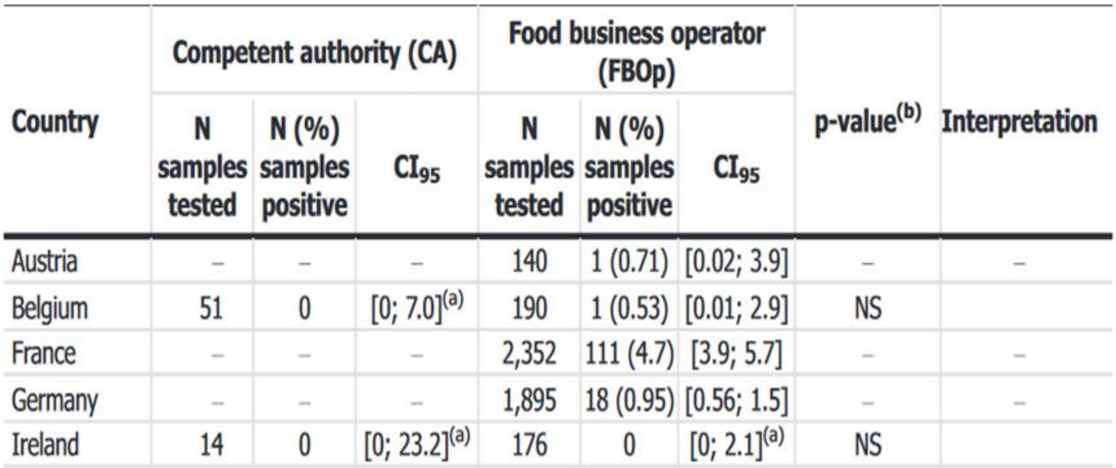
In countries with intensive poultry production, it has been determined that under current conditions it would be very difficult to eliminate ***Salmonella*** contamination in poultry production. However, the possibility to eliminate host specific serovars and to reduce non-host specific invasive serovars (paratyphoid) is realistic (Hafez, 2005).

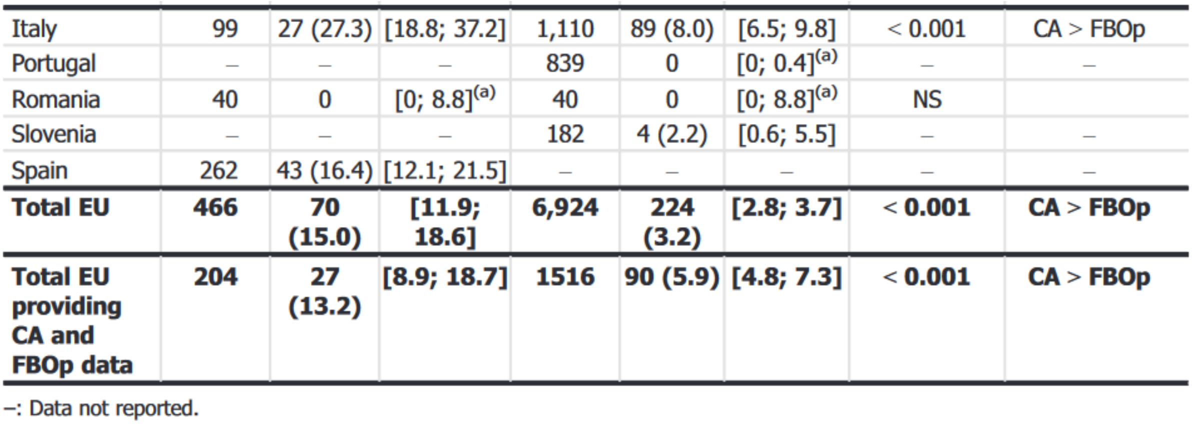
In June 2008 commission regulation (EC) No 584/2008 of implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards a community target for the reduction of the prevalence of **S. Enteritidis and S. Typhimurium** in turkeys was put into force (EC, 2008). The Community target is the reduction of both salmonella serovars to the maximum percentage of fattening and breeder flocks remaining positive to 1 % or less by 31 December 2012. The testing scheme necessary to verify progress in the achievement of the Community target is set out in the Annex of this regulation and should apply from 1st January 2010 **(Table 2).**

Table 2: The minimum sampling requirements

Zoonoses or zoonotic agent	Animal population	Time of sampling by food business operators
Breeding turkeys (EC, 2008)		
S. Enteritidis, *S. Typhimurium*	- rearing flocks	- day-old chicks - four-week-old birds - two weeks before moving to laying phase or laying unit
	- adult flocks:	- at least every third week during the laying period at the holding or at the hatchery
Fattening turkeys (EC, 2008)		
S. Enteritidis, *S. Typhimurium*	- turkeys	- within three weeks before the birds are moved to the slaughterhouse

Table 3: Comparisons of proportions (%) of *Salmonella*-positive single samples from turkey carcasses (neck skin samples after chilling, by sampler reporting MS, EU, 2020





Country	Competent authority (CA)			Food business operator (FBOp)			p-value[b]	Interpretation
	N samples tested	N (%) samples positive	CI_{95}	N samples tested	N (%) samples positive	CI_{95}		
Austria	–	–	–	140	1 (0.71)	[0.02; 3.9]	–	–
Belgium	51	0	[0; 7.0][a]	190	1 (0.53)	[0.01; 2.9]	NS	
France	–	–	–	2,352	111 (4.7)	[3.9; 5.7]	–	–
Germany	–	–	–	1,895	18 (0.95)	[0.56; 1.5]	–	–
Ireland	14	0	[0; 23.2][a]	176	0	[0; 2.1][a]	NS	
Italy	99	27 (27.3)	[18.8; 37.2]	1,110	89 (8.0)	[6.5; 9.8]	< 0.001	CA > FBOp
Portugal	–	–	–	839	0	[0; 0.4][a]	–	–
Romania	40	0	[0; 8.8][a]	40	0	[0; 8.8][a]	NS	
Slovenia	–	–	–	182	4 (2.2)	[0.6; 5.5]	–	–
Spain	262	43 (16.4)	[12.1; 21.5]	–	–	–	–	–
Total EU	**466**	**70 (15.0)**	**[11.9; 18.6]**	**6,924**	**224 (3.2)**	**[2.8; 3.7]**	**< 0.001**	**CA > FBOp**
Total EU providing CA and FBOp data	**204**	**27 (13.2)**	**[8.9; 18.7]**	**1516**	**90 (5.9)**	**[4.8; 7.3]**	**< 0.001**	**CA > FBOp**

–: Data not reported.

Campylobacter infections are now the leading cause of human bacterial gastroenteritis in many developed countries. In human Campylobacteriosis are steadily increasing and have already exceeded the number of salmonellosis cases in some EU countries.

Campylobacter infections in humans are mainly transmitted via contaminated food. No evidence has been found either for vertical transmission or for horizontal transmission from one flock to the next via persistent house-contamination. However, since the organism has been detected in the intestines of most slaughtered poultry, the major route for campylobacter contamination of poultry appears to be the horizontal transmission from the environment. Specific flocks that become infected show rapid rate of intra-house transmission and a high isolation rate from caecal swabs, water and litter. Investigations indicated that the external campylobacter load per bird is increasing during transport, de-feathering and evisceration, and decreasing at the other processing steps studied, with an overall reduction of the mean load from production-to-consumption of about 4 to 5 logs. Good hygienic practice protocols should be prepared and strictly followed in all stages of production. Biosecurity should be improved throughout the production chain. Since campylobacter is found in the environment, hygienic barriers should be constructed to keep them outside the house (Anderson *et al*., 2003). Controlling these foods-borne organisms requires beside legislations a broader understanding of how microbial pathogens enter and move through the food chain, as well as the conditions that promote or inhibit growth for each type of organism. Good hygienic practice protocols should be prepared and strictly followed in all stages of production. Biosecurity should be improved throughout the production chain. Since campylobacter found in the environment, hygienic barriers should be constructed to keep them outside the house.

Table 4: Summary of *Campylobacter* statistics related to humans and major Food categories. (EU, 2015 - 2019) *

	2019	**2015**	**Data source**
Humans			
Total number of confirmed cases	220,682	232,226	**ECDC**
Total number of confirmed cases/100,000 population (notification rates)	59.7	63.0	
Number of reporting MS	28	27	
Infection acquired in the EU	109,930	112,808	
Infection acquired outside the EU	6,513	6,444	
Unknown travel status or unknown country of infection	104,239	112,974	
Number of food-borne outbreak-related cases	1,254	1,483	**EFSA**
Total number of food-borne outbreaks	319	397	
Food			
Meat and meat products			
Number of sampling units	58,050	16,752	**EFSA**
Number of reporting MS	**24**	21	

*- ECDC: European Centre for Disease Prevention and Control; EFSA: European Food Safety Authority; MS: Member State.

Changes in social, political and consumer perceptions:

Antibiotic resistant and associated problems

The development of **antibiotic resistance** in bacteria, which is common in both, animals and humans, is and will also be a continue to be a never-ending public health hazard. Controlling these foodborne organisms requires a broader understanding of how microbial pathogens enter and move through the food chain, as well as the conditions that promote or inhibit growth for each type of organism.

It is generally known, that supplementation of poultry feed with antibiotic growth promoters (AGPs) improves performance of livestock. The effect of AGP on gut flora results in improvement of digestion, better absorption of nutrients, and a more stable balance in the microbial population. As consequence, the prevalence and severity of intestinal disorders are reduced. However, AGPs also can increase the prevalence of drug-resistant bacteria. Based on „Precautionary Principle" and experiences made in some European countries, the EU completely banned the use growth-promoting antibiotics in feed of food producing animals by January 2006. Field observations in Europe showed that the poultry industry faced several problems after the ban of AGPs. The impact of the ban has been seen on the performances (body weight and feed conversion rate) as well as on the rearing husbandry (wet litter and ammonia level), animal welfare problem (footpad dermatitis) and general health issues on the birds (enteric disorders due to dysbacteriosis and clostridial infections)**.** Investigations indicate that competitive exclusion, prebiotics, probiotics, enzymes, and acids can impact the incidence and severity of clostridial infections in poultry. According to Langhout (2007) these approaches will need adaptations in the feeding program and/or feed production. The practical relevance of these approaches may vary between the different areas in the world. At this moment it is difficult to evaluate novel strategies developed to antibiotic-free feeding concepts. Combination of different approaches is necessary, to enhance the performance and health status of the birds such as: selection of highly digestible feed ingredients to reduce nutrients for microbial degradation; introduction of a special pre-starter diet in the feeding program; improvement of climate control in the poultry house to avoid stress in the animal and keeping litter quality in optimal condition.

Multi-resistant bacteria are increasingly posing a hazard to human and animal health worldwide, impeding successful antibacterial treatment (Arias et al., 2010). In addition, the development of novel antibiotics does not keep step with the emergence of antimicrobial resistance in bacteria (García-Rey, 2010). Increasing application of antibiotics for the treatment of humans and animals and the use of the glycopeptide, avoparcin in subtherapeutic levels as a growth promoter in the past have been generally held responsible for a progressive deterioration of the resistance situation in bacteria (Aarestrup, 2005; Alanis, 2005). Among multi-resistant bacteria, vancomycin-resistant enterococci (**VRE**) have been estimated as one of the most common bacteria causing a rise in cases of nosocomial infections in humans in the last few years (Arias et al., 2010). The prevalence of vancomycin-resistant enterococci (VRE) in 20 turkey flocks reared in the southwest of Germany was recently investigated (Sting et al., 2013). The **VRE** could be isolated by means of a procedure combining bacterial cultivation in an enrichment broth and on a selective solid media. Enterococci were identified biochemically and subsequently tested on the presence of the vancomycin resistance genes vanA, vanB (B1/B2/B3), and vanC (C1/C2/C3) using real-time PCR assays. Vancomycin-resistant enterococci were detected in 15 (75%) of the 20 turkey flocks investigated. In 5 flocks, all animal samples and environmental dust samples taken were VRE-negative. In a total of cultivated 68 isolates from birds and dust samples, enterococci bearing *van*-genes were detected. Of these, 12 isolates carried the *vanA* gene (17.6%) and 56 isolates carried the *vanC1* gene (82.6%). Neither *vanB* (*B1*, *B2*, *B3*) genes nor the *vanC2* or *vanC3* genes could be detected.

In addition, Livestock-associated methicillin-resistant ***Staphylococcus aureus*** (LA-MRSA) has been isolated from a number of livestock species and persons involved in animal production. Infections with MRSA often prove difficult and expensive to treat. During the last few years LA-MRSA have been isolated from a number of farm animal species including pigs, veal calves, dairy cattle and broilers (De Neeling, et al. 2007; Hasman et al., 2010, Persoons et al., 2009). In addition, turkey meat was shown to be contaminated with MRSA (De Boer et al., 2009), however, only limited information is available concerning the situation in turkey flocks with respect to prevalence, site of colonization or infection and involved strains. Richter et al. (2012) investigated the prevalence of LA-MRSA in fattening turkeys and people living on farms that house fattening turkeys. Eighteen (90%) of 20 investigated flocks were positive for MRSA. All female flocks were positive, while 8 male flocks were positive. On 12 of the farms 22 (37.3%) of 59 persons sampled were positive for MRSA. None of them showed clinical symptoms indicative of an MRSA infection. People with frequent access to the stables were more likely to be positive for MRSA. In most flocks MRSA that could be assigned to clonal complex (CC) 398 were detected. In five flocks MRSA of spa-type t002 that is not related to CC398 were identified. Moreover, other methicillin-resistant Staphylococcus spp. were detected on 11 farms and in eight people working on the farms.

In Europe the Commission regulations 2205/2001 and 1798/1995 have stopped the usage of dimitridazoles as therapeutic or prophylactic drugs in food producing animals (EC, 1995, 2001) and council regulation1756/2002 banned the application of Nifursol from 31 March 2003 (EC, 2002). Following a ban of all previously used products for prophylaxis or treatment of histomonosis in the EU, is becoming increasingly responsible for considerable economic problems for the turkey industry, causing disastrous economic losses. A large number of outbreaks in turkey and layer flocks with varying mortality were reported in several European countries.

Changes in social, political and consumer perceptions

Animal welfare

Currently, there is great concern that serious animal welfare and health problems might have been caused already due to genetic selection practices within the turkey industry. Fact is that genetic selection practices within the turkey industry have achieved significant progress in terms of growth rate, better feed conversion, better meat yield and low production cost. All the time this was accompanied by continuous improvement in husbandry practices, nutrition, and disease control. The most outstanding and visible changes in modern turkey compared to their ancestors is **the rapid growth and the higher percentage of breast muscle**. As a consequence, it is important to understand the relationship between genetic selection pressures and other factors that may have a subsequent **impact on the health conditions**. This includes the effects on the growth and development of supporting structures such as bone and blood supply. Compared to traditional lines the highly selected birds have reduced cardiopulmonary capacity in relation to their muscle mass, as the ratio of heart and lung development has strongly decreased in modern turkeys. In addition, it is known that the blood pressure of the traditional turkey is only about half the value of that of the modern one (Norci and Montella, 2003; Havenstein et al., 2004). This physiological imbalance causes problems such as sudden death syndrome, aortic rupture, deep pectoral myopathy and skeletal disorders, which currently receive a lot of attention as a cause of concern from the animal welfare point of view and are mostly accompanied by economic losses for turkey meat producers due to mortality, predisposition to cannibalism, retardation of growth, increase of the condemnation rate and downgrading at the processing plant. The most common problems of the musculoskeletal system related to fast growth comprise dyschondroplasia and footpad dermatitis. The incidence and severity of both can be influenced by nutrition and genetic selection (Farquharson, and Jefferies, 2000; Mayne, 2003).

According to a published report on a new Animal Health Strategy for the European Union (2007 - 2013), the concept of animal health not only covers the absence of disease in animals, but also the relationship between the health of animals and their welfare. It will also take into account social, economic and ethical considerations, as well as support the achievement of a high level of environmental protection (EC, 2007).

Table 5: Some problems associated with genetic selection and rearing management in fattening turkeys (Hafez, 2015, Hafez and Shehata, 2020)

Disease conditions	**Some possible causes**	
	Non-Infectious	**Infectious**
Sudden death, Ascites	**Genetic:** Rapid growth, Reduced oxygen (hypoxia) **Management:** Stress, Excitement	**Not known**
Peri-renal haemorrhage	**Genetic:** Rapid growth, High blood pressure of some birds. **Management:** Stress, Continuous lighting programs, Hyperactivity	
Aortic rupture	**Genetic:** Rapid growth, High blood pressure of some birds. **Nutrition:** Copper deficiency, High fat and /or protein content. **Management:** Stress, Stocking density, Disquietness	
Deep pectoral myopathy	**Genetic:** Rapid growth, Inadequate vasculature in muscles of meat-type birds. **Management:** Traumatic, Multiple wing flapping, Excitement.	
Dyschondroplasia	**Genetic:** Rapid growth, high body weight. **Nutrition, Management**	
Leg disorders	**Genetic:** Rapid growth, **Nutrition, Management: Litter quality, Stocking density, Lighting program, Exercise, Deviation in hatching** **Reovirus, Mycoplasma, E. Coli, P. multicide ORT, Strept., Staph., Aspergillus**	**Several infectious agents**
Podo-dermatitis	**Genetic and Sex,** **Management:** Stocking density, litter material, quality and management, Humidity	**Not known**
Breast blisters	**Genetic:** Rapid growth, Slow feather development. **Management:** Litter quality, Stocking density, Mechanical- traumatic	Mycoplasma *Staph. aureu*

Emergence and Re-emergence of turkey diseases

Beside several managements related and political issues, emerging and re-emerging diseases and/or infections of turkeys are and will remain a continuous challenge for poultry veterinarians and the turkey industry.

An emergency animal disease (EAD) can be defined as a disease condition that is likely to have a significant effect on livestock - potentially resulting in livestock deaths, production losses and in some cases impacts on human health and the environment (Geering et al., 1999). Morse (1995) defined human emerging diseases as infections that either have newly appeared in a population or have already existed, but are rapidly increasing in incidence or geographic range. In general, most of emerging infections appear to be caused by pathogens already present in the environment that acquired a selective advantage or afforded an opportunity to infect new host populations. Moreover, there are other animal health emergencies that may be caused by non-infectious agents such as chemical residue problems in livestock or food safety problems through contamination of animal products. Several factors can precipitate and/or predispose to disease emergence. These include changes in the structure and development of the turkey industry, strong global competition and varying production costs in different countries and continents, leading to an increase of the global movement of the birds and products. This could also increase the risk to introduce a new infection to areas, which were previously considered to be free from such diseases (Hafez, 2009). Re-emerging and resurging infections are those that existed in the past but are now rapidly increasing either in incidence or in geographical or host range.

Health disorders and infectious diseases of turkeys are mostly associated with severe economic losses. Several pathogens are incriminated as possible causes of many disease complexes of turkeys either alone (mono-causal) or in synergy with different other micro-organisms (multi-causal) or accompanied by non-infectious factors. "**Non-infectious**" means all factors which influence the bird health and include house structure, climatic conditions (ventilation, temperature, and litter condition), stocking density, feed and water supply, hygienic condition as well as the knowledge and qualification of the stockman. These factors affect each other and can promote or inhibit the health condition of the flock. In aim to achieve desired performance results, managers of turkey flocks should integrate good environment, husbandry, nutrition and disease control programs. The rearing management must be directed to satisfy the bird's requirements, to promote the production and to prevent diseases condition. Any disturbance will cause stress, which will reduce the resistance of the birds, increase their susceptibility to infections and reduce their immune-response to vaccines.

Infectious diseases caused by several infectious agents such as viruses, bacteria, fungus and parasites are involved in many disease conditions. These infectious agents can be introduced and spread in turkey farms by different routes. It occurs by vertical and/or horizontal route. At early days of age, the main disease problems are related to vertically transmitted infections and improper hatchery eggs sanitation (Yolk sac infection/ Omphalitis) with salmonella, *E. coli*, Mycoplasma, Aspergillus, Staphylococci, Streptococci, Pseudomonas and Avian encephalomyelitis. Those and other infectious agents can also be transmitted horizontally (laterally) by direct contact between infected and non-infected birds. Currently, the most important problems of turkeys are **respiratory diseases**, possibly caused by avian Influenza, Newcastle disease virus, Avian-metapneumovirus (TRT) and /or *Ornithobacterium rhinotracheale*, Mycoplasma, *E. coli*., Aspergillus. Furthermore, enteric disorders caused by several viral agents such as coronavirus, astrovirus, rotavirus, Salmonella, Campylobacter or due to parasitic infestation such as coccidia and *Histomonas melagridis* are common problems.

The severity of clinical signs, duration of the disease and mortality are extremely variable and are influenced by kind, virulence and pathogenicity of the infectious agent as well as by many environmental factors such as poor management, inadequate ventilation, high stocking density, poor litter conditions, poor hygiene, high ammonia level, concurrent diseases and the type of secondary infection.

Future expectations Disease diagnosis, treatment and control

In future improvements in laboratory diagnosis, such as diagnostic micro array and other technologies, will allow faster, more sensitive and more accurate diagnosis of infectious diseases, and early interventions will become a reality.

However, only a few authorised **pharmaceutical veterinary products** will be available for the treatment of poultry as food producing animals. Future scientific findings on the pathogenic mechanisms of bacteria will help to improve the treatment of bacterial infections, and instead of non-specific antibiotic therapy, new drugs will be able to target the signalling mechanisms, which are able to disrupt the pathogenic effects of the pathogen bacteria.

Vaccination is regarded as one of the most beneficial biopharmaceutical interventions due to its ability to induce protection against infectious diseases via targeted activation of the immune system. Many valuable new vaccine production technologies have been developed and are in used. The use of future progressive vaccine production technologies, such as recombinant, subunit, reverse genetic and nucleic acid vaccines, can significantly reduce the cost of vaccines, ensure better efficacy, and allow easy and rapid intervention to face the steady mutation of the microorganisms. Furthermore, the development of efficient vaccines against bacterial infections will lead to a reduction of the use of antibiotics and subsequently of the development of resistant bacteria. **Genetic resistance and selective breeding:** to improve production traits and health is a long-standing goal of the industry. The desire to enhance breeding strategies through the use of molecular techniques (genetic linkage maps) will lead to the characterisation of genome structure and genes that are associated with production traits and disease susceptibility and resistance. This will allow selecting bird lines that are genetically resistant to several pathogens. In addition, **improvement of rearing technology, management and nutrition** will help to maintain bird comfort (Hafez 2009).

Conclusions

In the future, the global cooperation and trade will enforce the governments to harmonize the existing different legislations related to trade, animal disease control, animal nutrition as well as the licensing of drugs and vaccines for veterinary use.

In addition, the consumer expectations for high standards quality of poultry products will strongly influence the production methods. This means that farmers, veterinarians, stockholders and all other partners involved in the production chain will have to share more responsibilities and that cooperation will be intensified.

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Ingestion and digestion

Fibres: challenges and opportunities for the poultry industry (A. Moss)

Amy MOSS

Amy F. Moss[1], Natalie K. Morgan[1], M. Choct[1] [1] School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia

Corresponding author: Dr Amy Moss

Email: amoss22@une.edu.au

Abbreviated title: Fibres: challenges and opportunities

Summary

To achieve an optimum poultry performance, or output, we must have a thorough understanding of our inputs. This includes accurate feed ingredient characterisation. However, confusing definitions of dietary components have meant that there are substantial portions of our diets that are not at all accounted for in formulation. One of the most confused and poorly defined nutrients is fibre. Throughout the years, the definition of chemical fibre has evolved from Henneberg and Stohmann's 1859 crude fibre definition to Van Soest's detergent fibre system and finally to the dietary fibre system that classifies individual non-starch polysaccharides (NSP) and lignin. To further complicate, the chemical definition of fibre is also confused with structural fibre, with which particle size largely determines the attained benefits. Despite the development of the dietary fibre system, crude fibre is still routinely used in feed formulation and poultry nutrition. This is problematic as it has been calculated that 24% of the dietary components in Soybean meal are unaccounted for or 'missing' under the crude fibre system (Choct et al., 2015). Additionally, NSP data are often lacking from feed ingredient databases, or can be extremely variable (Moss, 2020). Therefore, there is vast opportunity to make fundamental changes in the way we distinguish and perceive fibre to improve poultry diet precision. The complete adoption of 'dietary fibre' to eliminate missing dietary components, development of more robust and region-specific databases of dietary fibre and determination of its variability within ingredients is our next challenge towards the complete characterisation of poultry diets to enhance poultry production.

Key words: Fibre, feed, ingredient, formulation, variation

Introduction

To achieve an optimum poultry performance, or output, we must have a thorough understanding of our inputs – feed ingredients. This is particularly important in recent years as many nutritionists strive for the precision feeding of poultry; maximising the contribution to nutrition and performance from each dietary component. However, to achieve this, accurate nutrient characterisation of all dietary components must first be attained. This is difficult in practice due to the costly and time-consuming nature of wet chemistry analysis, and is often confused further by overlapping or limited definitions of some nutrients. Thus, there are substantial portions of our diets that are not well represented or accounted for in diet formulation. One such nutrient which is rarely analysed and is likely the most confused and poorly defined is fibre.

Throughout the years, the definition of chemical fibre has evolved from Henneberg and Stohmann's 1859 crude fibre definition to Van Soest's detergent fibre system and finally to the dietary fibre system that classifies individual non-starch polysaccharides (NSP) and lignin. To further complicate, the chemical definition of fibre is also confused with structural fibre, with which particle size largely determines the attained benefits. Today, dietary fibre is a complete definition, consisting of the sum of NSPs and lignin (Figure 1).

Dietary fibre is of importance in poultry nutrition, and NSPs are often in focus largely due to the effect on solubility and viscosity. NSP are a necessary component of plant cell walls; however, in the context of animal feed, soluble fibre may increase viscosity of digesta throughout the small intestine thereby providing a mechanical barrier to nutrient digestion by reducing enzyme access to substrate (Kim et al., 2005) and absorption by limiting transport of nutrients to the intestinal mucosa (Slominski, 2011). While less discussed, NSP may also have nutrient encapsulating effect, forming strong cross-linkages reinforcing cell walls and preventing enzyme access (Edwards 2010). As a consequence, the beneficial effects of including NSP-ases are well documented. For example, supplementation of low metabolisable energy wheat (12.02 MJ/kg) with glycanase reduced digesta viscosity, enhancing nutrient absorption which led to an improvement in AME to 14.94 MJ/kg, and improved weight gain by 30%; matching that of the normal metabolisable energy wheat (Choct et al., 1995).

Despite the importance of considering dietary fibre in feed formulation and the implications it has for poultry performance, there are still challenges to overcome before the concept of dietary fibre may be fully adopted in feed formulation. Therefore, the aim of this review is to highlight some of the challenges to implementing dietary fibre within feed formulation and highlight the opportunities available to improve poultry feed formulation and move closer to the goal of precision nutrition.

Challenges

Despite the development of the dietary fibre system, crude fibre is still routinely used in feed formulation and poultry nutrition. This results in a ‘nutrient gap’ as with the complete characterisation of poultry diets, it is evident that as much as 24% of the dietary components in Soybean meal have been unaccounted for or ‘missing’ due to inadequate chemical definitions such as the crude fibre system (Choct et al., 2015). Over the entire diet, this unaccounted-for fibre can represent up to 10% of the diet (Choct, 2015); a substantial portion to be omitted. This is particularly troublesome in regards to selecting NSP-ase enzymes for inclusion to poultry diets and during research on these enzymes; if the substrate is not well defined, how can the response of the enzyme be accurately measured or predicted? Despite the uncertainty the crude fibre system creates in diet formulation, it is likely still in use due to the practicalities of NSP determination in feedstuffs.

Variability of NSP within feed ingredients

One likely reason for the persistence of the crude fibre measurement is the ease at which crude fibre may be determined compared to the lengthy procedure and expense of the complete wet chemistry characterisation of dietary NSP and lignin, which is impractical in an industry setting. An alternative option to wet chemistry analysis for industry nutritionists is to provide industry with robust dietary fibre matrices or calibrations of common feedstuffs. Presently, within Australia, a database of dietary fibre contents of common Australian feed ingredients is available (Non-Starch Polysaccharide Database, 2020). While this database provides a comprehensive view of the NSP contents of several feed ingredients, the database reveals that there is a substantial amount of variation present within the NSPs of Australian feed ingredients and thus more samples should be tested to improve the accuracy by which the mean may be estimated (Moss et al., 2020). Possible reasons for this range in NSP values include the broad range of varieties, seasonal effects and growth sites across Australia’s vast continent (Hughes and Choct, 1999). For example, a total of 48 Australian wheat samples were scanned via near infra-red spectroscopy (NIR) to determine their protein concentration (Little and McGrath, 2019). It was reported that protein levels had decreased over the 2018/19 harvest compared to the 2017/18 harvest, having reduced by 23%. Additionally, protein level varied by year but also location across Australia. Similarly, the same variation may be seen in NSP concentration as soluble NSP and total NSP varied over just one year of harvest (1992-1993) in Choct et al. (1999), by as much as 9% and 2.4%, respectively.

Additionally, the processing of feedstuffs may also alter NSP concentrations. For example, corn, wheat and mixed-cereal DDGS samples were analysed for protein, fibre, fat, mineral and NSP content in Pedersen et al. (2014). Among the DDGS samples reported in this study, the most variable nutrient measured was soluble-NSP content, with particular mention to soluble arabinoxylan. It was determined that this variability may be attributed to modification of the endosperm arabinoxylan during the fermentation and drying process. Due to this variability, considerable investment would be required in order to generate large enough sample numbers for each ingredient to accurately estimate the mean and distribution. This also complicates the reliability of NSP matrices and predictions such as NIR calibrations. However, considering that up to 10% of the feed may be unaccounted for under a crude protein measurement and feed constitutes 60% of the overall production cost; this is an important future field for development and there is vast opportunity to make fundamental changes in the way we distinguish and perceive fibre to improve poultry diet precision.

Rapid assessment of NSP

Due to the wide range of values for NSP that may be contained within ingredients, this presents a challenge for feed formulation as analysis of NSP levels is costly and time consuming but the inherent variability of NSP within ingredients means it is difficult to accurately predict. Thus, development of an NIR NSP calibration would likely be the most adaptable and accurate method of assessing NSP concentration within an industry setting (Blakeney and Flinn, 2005). Previously, Hollung et al. (2006) evaluated the efficacy of NIR to determine NSP and oligosaccharide contents of soybean meal. Samples were limited and thus a strong conclusion could not be drawn; however, correlations are encouraging (total NSP $r = 0.91$, sample number = 12). Additionally, correlations between total soluble and insoluble NSP concentrations and NIR were reported in Blakeney and Flinn (2005). While more samples are examined with sample numbers of 71 for both total soluble and insoluble NSP, unfortunately they are not from a specific source and are a combination of grains and pulses. Nevertheless, the strong correlations in total insoluble NSP ($r^2 = 0.89$) and total soluble NSP ($r^2 = 0.68$) was promising.

Development of commercial NSP NIR calibrations is in focus and some commercial NSP calibrations are now available (AUNIR, 2015; Smeets and Nuyens, 2016). Such calibrations will likely become more widespread as the cost of feed continues to increase.

Feed Formulation

While NIR presents a relatively cheap and quick method to assess NSP content of feedstuffs, it is not practical in all situations as nutritionists within large integrated companies may experience delays in receiving NIR information and many consultant nutritionists do not have access to an NIR system. Thus, many nutritionists may still rely on historical or ‘book’ values. This can be problematic due to the inherent variability of NSP. Nevertheless, techniques such as stochastic feed formulation may be employed to formulate diets with greater certainty that NSP levels fall within predicted ranges, which would help nutritionists with decisions; for example, the most appropriate enzyme cocktail.

Feeds formulated on average values are expected to contain less than the minimum restriction 50% of the time (Pesti and Seila, 1999). This may hold particularly important consequences for NSP levels as the deviation from the mean of some samples is quite large, meaning there is more chance of attaining a value that may have practical consequences. For example, to examine the variability in NSP content that may occur within the typical Australian diet; starter, grower, finisher and withdrawal diets were formulated to represent standard modern Australian broiler diets (Table 1). From these diets, the typical inclusion of non-starch polysaccharide containing feed ingredients to Australian broiler starter, grower, finisher and withdrawal diets may be deduced and thus the expected contribution to the total dietary NSP standard deviation was calculated (Table 2). The expected standard deviation of total NSP of feed ingredients was obtained from the Australian Non-Starch Polysaccharide Database (2020). As the expected standard deviation and mean total NSP levels have been reported, simulations can be performed (assuming normality) to estimate the range of values and likelihood of attaining an extreme value in a diet formulated using the mean value. This was performed for the following example using Microsoft Excel 2016, NORMINV function, with 10,000 individual simulations per diet.

Simulations were performed for starter, grower, finisher and withdrawal diets (Figures 2a, b, c and d respectively). Dietary total NSP contents of 141.1 g/kg or above are reported to significantly depress weight gain and 35-day body weights (Senthilkumar and Balakrishnan, 2014). Thus, in this simulation we will use a dietary total NSP contents of 141.1 g/kg as the threshold beyond which practical implications may occur. Within a starter diet formulated using mean total NSP values, there is roughly a 16.6% chance (or approximately 1 in 5 odds) of the actual dietary total NSP values falling above 141.1 g/kg total NSP. Similar odds are expected from the grower, finisher and withdrawal diets as they possess very similar distributions. Thus, a nutritionist may formulate a diet using book values and expect a total NSP level of 112 g/kg and likewise select an enzyme cocktail for this value. However, there is a substantial chance that total NSP levels will be much higher and thus the enzyme included may not be adequate, and performance issues may result. Thus, it is in this way that stochastic feed formulation is an important and informative decision-making tool. Stochastic models quantify the level of uncertainty of ingredient nutrient variability that exists in feed formulation using the known standard deviation of the nutrient. With this information, the diet's nutrient distribution can essentially be shifted to allow a nutritionist to achieve a set level of probability that the diet will fall within the desired nutrient levels. As stochastic feed formulation determines the probability of attaining a certain nutrient level, it has been demonstrated to be more accurate than assigning safety margins alone and is thereby reported to be more cost effective (D'Alfonso et al., 1992). Nevertheless, a barrier which may be preventing the adoption of stochastic feed formulation within the poultry industry today is the inconsistent reporting of descriptive statistics such as sample number, standard deviation and distribution which exists across many databases (Moss et al., 2020). Information regarding the distribution is particularly important as ingredients may not necessarily follow a normal distribution. In this case, the mean alone may provide a poor estimate and thus the median value is a better determination of the true central tendency than the mean (Weiss, 2004). Without this important information, decisions as to the quality of the data, estimation of the probability of formulating a diet which has inadequate nutrient content or excess anti-nutrient, and the consequences of uncertainty within feed formulation cannot be accurately quantified.

Opportunities: the path towards precision nutrition?

Poultry nutrition is well on its way to achieving precision nutrition. However, one of the final challenges is the characterisation of dietary fibre as analytical methods are costly and time consuming and the NSP content of feed ingredients is highly variable. Due to the practicalities of analytical NSP determination, improved feed characterisation may be achieved via the adoption of commercial NSP NIR calibrations. Nevertheless, until NIR is accessible for all nutritionists (which will likely occur as technology becomes cheaper over time), book values will still be important and thus deserve greater attention to detail. This includes accurate sample reporting which must describe the mean and also the sample number, standard deviation and indication as to the type of distribution (normal or skewed) in order for nutritionists to make decisions on the reliability that the mean value may provide and use stochastic feed formulation techniques to predict the level of uncertainty that may exist within their formulation.

Conclusion

Fibre continues to be one of the most confused and poorly defined nutrients, and within feed ingredients, its concentration can be quite variable. This presents challenges for precision nutrition of poultry as a substantial proportion of the diet is unaccounted for and potentially under-utilised. The likely cause of these challenges is the expensive and time-consuming nature of NSP analysis. Nevertheless, there are many opportunities for the poultry industry to enhance the adoption of dietary fibre including the development of more robust and region-specific databases and NIR calibrations of dietary fibre and further determination of its variability within ingredients; including the standard deviation and characterisation of the distribution. With this additional information, feed formulation techniques such as stochastic formulation may help nutritionists to reduce the uncertainty that exists within poultry diets, and approach the complete characterisation of diets to enhance poultry production.

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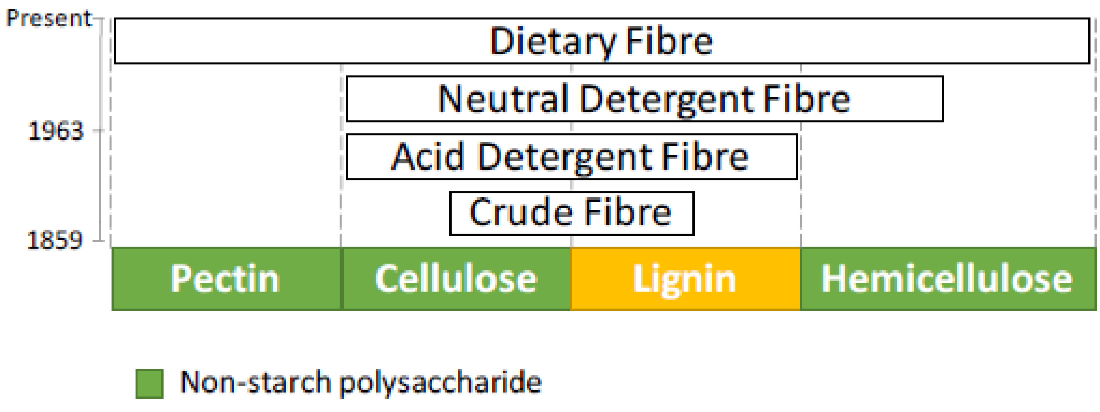


Figure 1 Diagrammatic representation of the different definitions of fibre and their chronology.

Table 1 Composition and nutrient specifications of starter (0-10 days post-hatch), grower (11-24 days), finisher (25-37 days) and withdrawal (38-42 days) diets formulated to the mean total non-starch polysaccharide (NSP) level.

Ingredient (g/kg)	Starter	Grower	Finisher	Withdrawal
Wheat	578.8	621.3	656.8	655.4
Soybean meal	319.1	258.0	205.1	207.8
Canola seed full fat	30.0	60.0	80.0	80.0
Oil (soy)	15.0	24.1	26.1	25.9
Tallow	13.6	-	-	-
Limestone (38% Ca)	13.4	12.0	10.6	10.6
Salt	1.93	1.75	1.77	1.77
Phosphate	8.60	6.80	4.98	4.95
Sodium bicarbonate	2.98	2.68	2.66	2.66
Betaine	1.30	1.30	1.30	1.30
L-lysine sulphate	4.76	4.42	3.93	3.81
DL-methionine	3.45	2.91	2.49	2.46
L-threonine	1.67	1.39	10.75	1.04
Choline Chloride (75%)	0.25	0.25	0.20	0.20
Vitamin and mineral premix[1]	4.50	2.50	2.50	1.5
Xylanase	0.25	0.25	0.25	0.25
Phytase	0.30	0.30	0.30	0.3
Nutrient (g/kg; unless specified)				
Total NSP	112.1	111.0	108.2	108.4
AMEn (MJ/kg)	12.55	12.97	13.39	13.39
Crude protein	228.0	209.0	191.0	192.0
lysine[2]	12.8	11.50	10.2	10.2
methionine[2]	6.28	5.58	5.00	4.98
methionine+cystine[2]	9.5	8.70	8.00	8.00
cysteine[2]	3.21	3.11	3.00	3.01
threonine[2]	8.6	7.70	6.80	6.80
tryptophan[2]	2.59	2.38	21.8	2.19
glycine [2]	7.67	7.04	6.45	6.49
arginine[2]	13.18	11.81	10.53	10.61
serine[2]	7.67	7.04	6.45	6.49
histidine[2]	4.91	4.49	4.08	4.11
isoleucine[2]	8.33	7.53	6.78	6.83
leucine[2]	14.26	12.97	11.76	11.84
valine[2]	9.05	8.31	7.59	7.64
phenylalanine[2]	9.39	8.48	7.65	7.70
ash	52.62	47.36	42.13	42.26
crude fat	56.73	63.08	72.17	72.02
calcium	9.60	8.70	7.80	7.80
total phosphorus	5.44	4.94	4.42	4.43
avail. phosphorous	4.80	4.35	3.90	3.90
sodium	19.5	1.80	1.80	1.80
chloride	2.00	1.90	1.90	1.90
potassium	9.55	8.70	7.91	7.96
electrolyte balance (mEq/kg)	272.7	247.3	227.0	228.3
choline (mg/kg)	1839.3	1783.9	1684.0	1691.9
Cost ($AUD)	452.90	437.30	429.60	418.63

[1] The appropriate vitamin and mineral premix (starter, grower, finisher and withdrawal) was substituted within each formulation. [2] Available.

Table 2 Typical inclusion of non-starch polysaccharide containing feed ingredients[1] to Australian broiler starter, grower, finisher and withdrawal diets, and standard deviation (SD).

Feedstuff	Approximate proportion of diet (%)	Total NSP level of diet (g/kg)	Total NSP supplied to diet (g/kg)	Proportionate SD of total NSP supplied to diet (g/kg)[2]
Starter diet		*112*		*34.25 (CV = 30.6)*
Wheat	57.8		60.7	22.2
Soybean meal (origin: Brazil)	31.9		48.2	11.5
Canola seed full fat	3.0		3.1	0.6
Grower diet		*110*		*34.28 (CV = 31.2)*
Wheat	62.0		*65.1*	23.8
Soybean meal (origin: Brazil)	25.8		39.0	9.3
Canola seed full fat	6.0		6.2	1.2
Finisher diet		*108*		*34.20 (CV = 31.1)*
Wheat	65.7		69.0	25.2
Soybean meal (origin: Brazil)	20.5		31.0	7.4
Canola seed full fat	8.0		8.2	1.6
Withdrawal diet		*108*		*34.23 (CV = 31.1)*
Wheat	65.5		69.0	25.2
Soybean meal (origin: Brazil)	20.8		31.4	7.5
Canola seed full fat	8.0		8.2	1.6

[1] Total NSP content of wheat = 105 g/kg (SD = 38.4); total NSP content of soybean meal = 151 g/kg (SD = 35.9); total NSP content of canola seed = 103 g/kg (SD = 20.1); means and SD sourced from the Non-Starch Polysaccharide Database (2020).

Ratites

Recent advances in the breeding of farmed ostriches (S. Cloete)

Schalk CLOETE

S.W.P. Cloete (1,2), A. Engelbrecht (1,2), M. Bonato (1), P.T. Muvhali (1,2), Z. Brand (1,2), and I.A. Malecki (3)

(1) Dept. of Animal Sciences, Stellenbosch University, Matieland, 7602 South Africa

(2) Directorate Animal Sciences, Western Cape Department of Agriculture, Elsenburg, 7607 South Africa

(3) School of Agriculture and Environment, Faculty of Science, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia

Corresponding author: schalkc2@sun.ac.za

Abbreviated Title: Ostrich breeding advances

Summary

This review covers genetic and environmental parameters for ostrich traits and traits combinations not previously reported. Genetic analyses on embryonic deaths indicated that derived heritability estimates were independent of the inclusion of the effect of evaporative water loss in the model. Updated analyses on skin traits with a non-normal distribution using threshold and linear-threshold analyses suggested that these traits were heritable on the underlying scale and not correlated with skin size. Analyses on temperament confirmed that intensive interactions with humans at a young age resulted in a more docile temperament in juvenile ostriches. Moreover, temperament traits were heritable and mostly uncorrelated with slaughter and skin traits. Preliminary heritability estimates for monthly egg production and average egg weight in single-penned females maintained to develop a protocol for the artificial insemination of ostriches were consistent with those derived for pair-mated birds.

Genetic correlations were thus derived between traits measured repeatedly in mature birds (live weight, egg production and chick production) and slaughter traits in their offspring (slaughter weight, skin size, nodule size score and hair follicle score). Adult weight was genetically positively correlated with slaughter weight, skin size and nodule size score. Reproduction traits of mature females were generally not correlated with offspring slaughter traits, barring an unfavourable genetic correlation of egg production with skin size. Further analyses across trait complexes should be prioritised for further studies. Important trait complexes not yet studied are feather traits in juvenile birds and resistance to external stressors associated with commercial ostrich farming systems. Information on all these trait combinations is needed to derive an index ensuring that ostrich breeding is conducted in an ethical and sustainable way.

Key words: Genetic parameters, incubation, skin traits, Threshold models, temperament, artificial insemination, trait complexes, future perspectives

Background

Previous reviews on the breeding of ostriches focused on challenges stemming from the communal breeding system of ostriches to the capturing of individual performance and pedigree records (see Cloete *et al*., 1998; 2002). The pair-mating breeding system, although imperfect, alleviated most of these challenges and it was demonstrated that key traits of economic importance were both variable and heritable (Cloete *et al*., 2008b). To further substantiate claims that it is feasible to genetically improve production traits in ostriches within the constraints of the pair-mating system, direct additive genetic gains have been demonstrated for live weight as well as for egg and chick production (Cloete *et al*, 2008a). Despite these reported gains in genetic parameter estimation and the demonstration of genetic gains, several gaps in the scientific knowledge around scientific ostrich breeding were identified (Cloete *et al*., 2008b). It is also worth mentioning that the current paper still almost entirely relies on research conducted in South Africa, in many cases with international collaboration, as also noted in our previous review (Cloete *et al*., 2008b). The only other known genetic study that we are aware of was done on live weight of Brazilian ostriches (Ramos *et al*., 2014). This study was characterised by a marked attrition of records from hatching weight (n=2858) to 180 days of age (n=567), but nevertheless reported significant genetic variation at all ages.

This background statement has shown that, although some progress has been made, there are still opportunities to further develop scientific ostrich breeding. Since the publication of the above-mentioned reports several advances in scientific ostrich breeding has been made. These will be highlighted under the headings that follow.

Genetic analyses on embryo and chick losses as sources of reproductive wastage

Ostrich embryo mortalities amount to large losses to the industry, with total mortalities of up to 28.5% of fertile eggs (Brand et al., 2007). The vast majority of eggs in commercial ostrich production systems are artificially incubated. The paucity in genetic parameters for incubation traits was addressed in a paper that showed useful genetic variation for evaporative water loss to 21 and 35 days of incubation (Brand *et al*., 2009). Genetic variation was also evident for early embryonic mortalities (before candling at 21 days of incubation), late embryonic mortalities (subsequent to candling at 21 days of incubation), as well as for overall embryonic losses when analysed as at trait of fertile eggs incubated (Brand *et al*., 2012). Threshold model analyses suggested that early embryonic survival was genetically correlated to late embryonic survival (r_g=0.78;

Brand, 2012). Selection for either chick production or live weight resulted in favourable correlated genetic responses in embryonic losses (Brand *et al*., 2012).

In the absence of large-scale reports on the effect of differences in evaporative water loss on embryonic mortalities in artificially incubated ostrich eggs, a more recent study modelled the regression of embryo mortality on evaporative water loss using either a second-degree polynomial or a cubic spline (Z. Brand, unpublished). It is known that embryo losses increase with increasing age in female ostriches (Brand *et al*., 2007; 2012). Given the known female age effects, water loss was interacted with female age. The general trends were quite robust across embryonic mortality traits and water loss traits, but overlapping standard errors complicated interpretation when all age groups were presented in a single graph. The effects of age and water loss to 35 days of incubation on late embryonic mortality is therefore presented in Figure 1a for 2-, 5-, 6- and 9+-year-old birds when using a second-degree polynomic approach to emulate the U-shaped relation of embryonic losses with evaporative water loss. Figure 1b contains a similar figure modelled with a cubic spline, allowing a more nuanced smooth curve. Female age markedly affected embryo losses with the lowest losses in eggs produced by 2-year-old females and the highest in females of 9 years and older, while 5- and 6-year-old females occupied an intermediate position. Furthermore, it was clear that eggs with intermediate evaporative water loss had the lowest embryo losses. Eggs with either low or high-water losses sustained markedly higher embryo mortalities, although the impact of excessive water loss appeared to be less severe in eggs produced by 2-year-old birds compared to the other age groups. It was noted that female age differences were less evident at lower water loss levels when the cubic spline was used. Moreover, the impact of low levels of water loss declined faster to reach appreciably lower embryonic losses at 10 to 11% water loss. Embryo losses only started to increase at water loss percentages of around 17 to 18%. Although embryo losses increased markedly up to a water loss percentage of 21%, the rate of the incline appeared to be slower than at low water loss percentages. It thus seems that the spline approach produced curves that more closely emulated the actual observed impact of water loss on embryo losses (Z. Brand, unpublished).

Single-trait heritability estimates using models accounting for the effect of evaporative water loss by either quadratic polynomials or cubic splines are provided relative to the baseline estimates for the three embryo mortality traits, namely early, late and total embryonic mortality (Table 1). When assessed relative to the corresponding standard errors, it could be concluded that heritability estimates for embryonic losses in ostriches were largely independent of whether the impact of evaporative water loss was accounted for or not. Likewise, accounting for water loss effects had no appreciable effects on the maternal common environmental and paddock variance ratios.

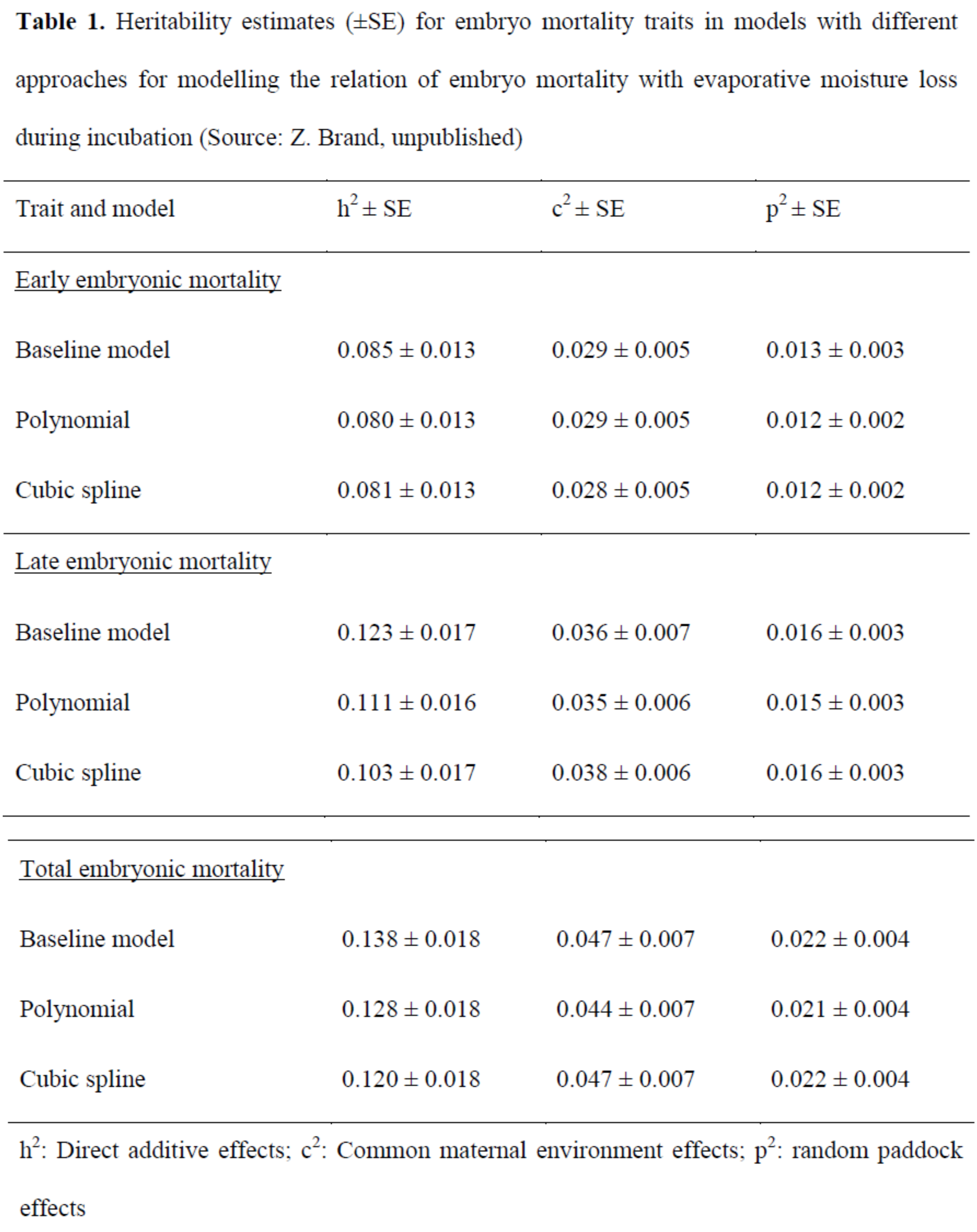


Table 1. Heritability estimates (±SE) for embryo mortality traits in models with different approaches for modelling the relation of embryo mortality with evaporative moisture loss during incubation (Source: Z. Brand, unpublished)

Trait and model	$h^2 \pm SE$	$c^2 \pm SE$	$p^2 \pm SE$
Early embryonic mortality			
Baseline model	0.085 ± 0.013	0.029 ± 0.005	0.013 ± 0.003
Polynomial	0.080 ± 0.013	0.029 ± 0.005	0.012 ± 0.002
Cubic spline	0.081 ± 0.013	0.028 ± 0.005	0.012 ± 0.002
Late embryonic mortality			
Baseline model	0.123 ± 0.017	0.036 ± 0.007	0.016 ± 0.003
Polynomial	0.111 ± 0.016	0.035 ± 0.006	0.015 ± 0.003
Cubic spline	0.103 ± 0.017	0.038 ± 0.006	0.016 ± 0.003
Total embryonic mortality			
Baseline model	0.138 ± 0.018	0.047 ± 0.007	0.022 ± 0.004
Polynomial	0.128 ± 0.018	0.044 ± 0.007	0.021 ± 0.004
Cubic spline	0.120 ± 0.018	0.047 ± 0.007	0.022 ± 0.004

h^2: Direct additive effects; c^2: Common maternal environment effects; p^2: random paddock effects

Ostrich chick mortality was also previously identified as an important constraint to efficient ostrich production (Cloete *et al*., 2008b). Studies on the genetic basis of chick mortality confirmed significant genetic variation, although the derived heritability estimates were low at 0.06 for the interval from hatching to 3 weeks and 0.05 for the interval from 4 to 12 weeks of age (Wang *et al*., 2011). Threshold model heritability estimates were somewhat higher at 0.06 to 0.12 for age specific chick survival (Wang, 2012). The absolute genetic correlations of age specific chick survival with hatching weight were positive at 0.16 to 0.24 in threshold linear models, but these correlations were not significant. The potential to improve chick survival by indirect selection therefore seems limited, although there appear to be scope for direct selection.

Genetic analyses on growth, slaughter and skin traits

The monetary income from slaughtered ostriches mostly depends on meat yield, skin yield and skin quality (Engelbrecht, 2013). The heritability of juvenile ostrich live weight increases with age at weighing (Engelbrecht *et al*., 2011), while genetic correlations exceeding 0.80 were reported among monthly live weights from 4 to 12 months of age. Ramos et al. (2014) reported heritability estimates of 0.16 for weight at 90 days and 0.24 for weight at 180 days of age, respectively.

Genetic variation was also demonstrated for all objectively measured skin traits (Engelbrecht et al., 2009b; Engelbrecht, 2013; Nemutandani, 2022), as well as the subjectively scored skin traits, namely nodule size, nodule shape, hair follicle prevalence and pitting damage (Engelbrecht *et al*., 2009a; Nemutandani, 2022). These traits were defined in detail in the review paper by Engelbrecht *et al*. (2009b).

Quantitative meat yield traits also exhibited significant genetic variation (Engelbrecht *et al*., 2013). Genetic correlations of slaughter weight and skin size with quantitative skin and meat traits were generally favourable and often high (Engelbrecht *et al*., 2013). Slaughter weight and skin size were also favourably correlated with subjective skin traits (Engelbrecht *et al*., 2009a; Nemutandani, 2022). The generally favourable genetic correlations among slaughter traits motivated Engelbrecht (2013) to model the relative change in monetary value under selection regimes incorporating different levels of information. It was shown that substantial monetary gains were still feasible in slaughter traits when only a single live weight record of the animal to be selected was available, a result of the above-mentioned favourable genetic correlations (Table 2).

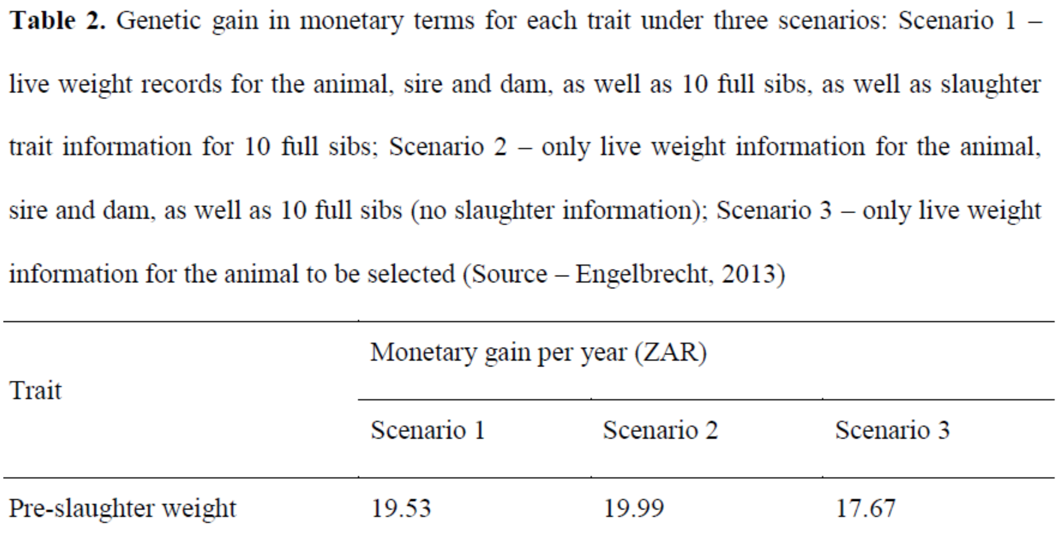


Table 2. Genetic gain in monetary terms for each trait under three scenarios: Scenario 1 – live weight records for the animal, sire and dam, as well as 10 full sibs, as well as slaughter trait information for 10 full sibs; Scenario 2 – only live weight information for the animal, sire and dam, as well as 10 full sibs (no slaughter information); Scenario 3 – only live weight information for the animal to be selected (Source – Engelbrecht, 2013)

Trait	Monetary gain per year (ZAR)		
	Scenario 1	Scenario 2	Scenario 3
Pre-slaughter weight	19.53	19.99	17.67

Skin size	14.38	13.83	12.22
Nodule size score	1.53	1.21	1.07
Nodule shape score	1.15	0.59	0.52
Hair follicle score	0.62	0.43	0.38
Total response	**37.20**	**36.05**	**31.86**

While results for the bulk of skin traits were thus favourable, there are several skin traits not subscribing to the assumptions for linear models' analysis. Five traits with a non-normal distribution were thus analysed by using threshold and linear-threshold methods (Nemutandani, 2022). These traits included outlier nodules (occurrence of an outlier nodule on the widest part of the crown, where 0 is allocated if it is absent, 1 for an outlier nodule on one side and 2 for outlier nodules on both sides), nodule direction (nodules in the crown area are generally facing caudally, with 0 being allocated if nodules are facing in different directions, while skins with nodules facing predominantly caudally are scored as 1) and nodule distribution (uniformity or diversity of nodule size, shape and distribution in the crown area, scored on a scale of 1 to 3, with 1 indicating poor distribution of all aspects and 3 indicating good distribution of all aspects of the nodules), prevalence of pitting damage (scored from 1 to 9, with 1 indicating no pitting damage and 9 excessive pitting damage) and the presence/absence of disappearing nodules on the binomial scale (0 = absent, 1 = some areas without nodules within the nodulated crown area). Discrete values modelled numbered 3 for nodule outlier score, 2 for nodule direction score, 3 for nodule distribution score, 5 for pitting score (collapsed from 9 categories) and 2 for the presence of disappearing nodules. Figure 2 depicts posterior distributions for the genetic and environmental components of pitting score as a threshold trait (a) and skin size as a trait with a normal distribution (b). The slightly skewed distributions for pitting and the symmetric distribution for skin size are evident.

The threshold traits were all heritable, with derived estimates ranging from 0.17 for nodule distribution score to 0.47 for nodule outlier count (Table 3). Significant (i.e. more than twice the standard error) genetic correlations occurred between the presence of nodule outliers and pitting score, while disappearing nodules was genetically correlated with nodule direction and nodule distribution scores. Further analyses confirmed that none of the subjectively assessed threshold traits were correlated with skin size as a quantitative linear trait (Nemutandani, 2022).

Feathers were once the primary trait of interest in ostrich production. However, the monetary value of feathers has dwindled, leading to a reduction in research on this trait complex. Feathers has become more important once more in recent years, requiring further research in the genetics of feather traits. Brand and Cloete (2009) reported that the heritability of the weights of different types of feathers ranged from 0.11 to 0.16, with the heritability of total feather weight estimated at 0.30. Genetic correlations among different feather weight categories varied in magnitude, ranging from moderate to very high (Brand and Cloete, 2009). A subsequent analysis across feather types yielded low but significant heritability estimates of 0.08 for feather length, 0.04 for feather width and 0.12 for feather weight (Brand and Cloete, 2015). A further upsurge in feather prices is likely to prompt further research on the genetics of feather traits.

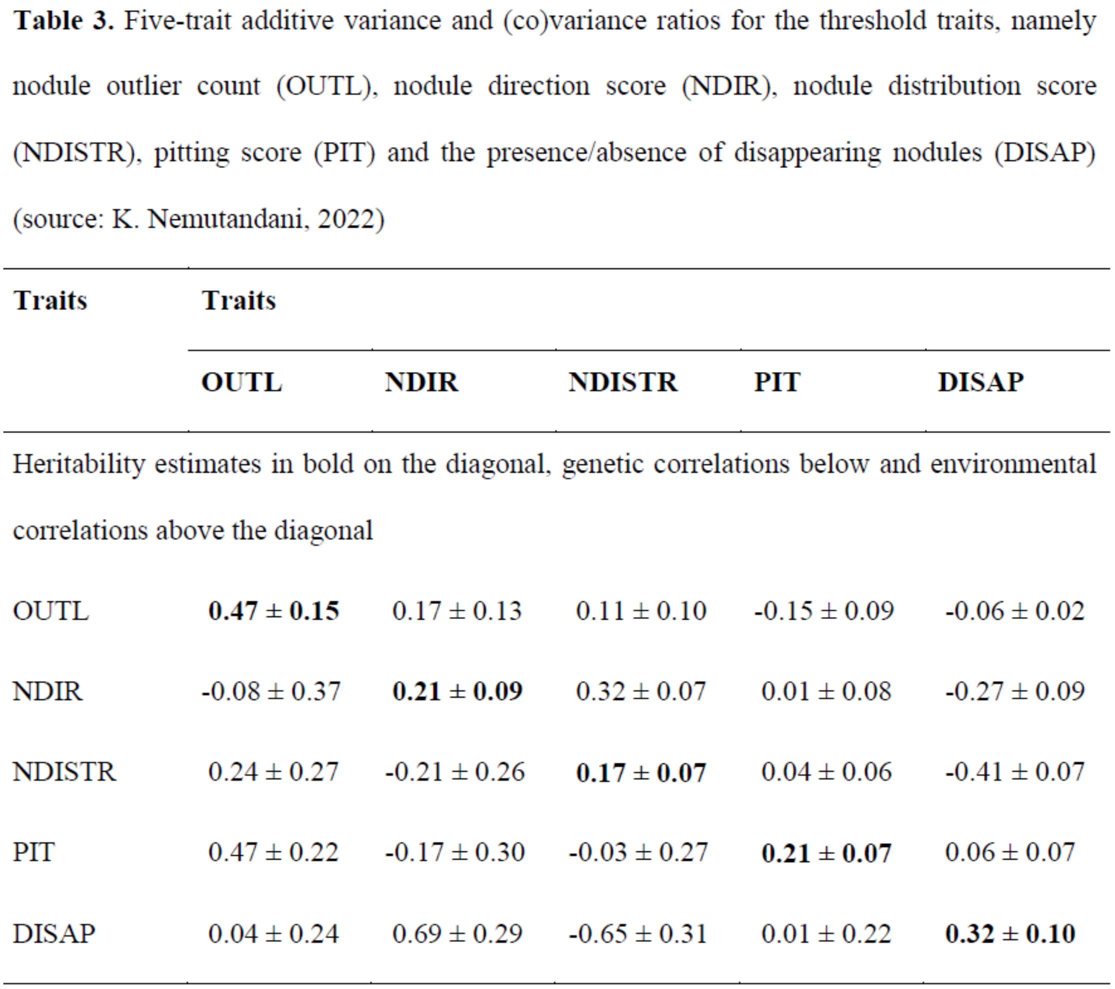


Table 3. Five-trait additive variance and (co)variance ratios for the threshold traits, namely nodule outlier count (OUTL), nodule direction score (NDIR), nodule distribution score (NDISTR), pitting score (PIT) and the presence/absence of disappearing nodules (DISAP) (source: K. Nemutandani, 2022)

Traits	**Traits**				
	OUTL	**NDIR**	**NDISTR**	**PIT**	**DISAP**
Heritability estimates in bold on the diagonal, genetic correlations below and environmental correlations above the diagonal					
OUTL	**0.47 ± 0.15**	0.17 ± 0.13	0.11 ± 0.10	-0.15 ± 0.09	-0.06 ± 0.02
NDIR	-0.08 ± 0.37	**0.21 ± 0.09**	0.32 ± 0.07	0.01 ± 0.08	-0.27 ± 0.09
NDISTR	0.24 ± 0.27	-0.21 ± 0.26	**0.17 ± 0.07**	0.04 ± 0.06	-0.41 ± 0.07
PIT	0.47 ± 0.22	-0.17 ± 0.30	-0.03 ± 0.27	**0.21 ± 0.07**	0.06 ± 0.07
DISAP	0.04 ± 0.24	0.69 ± 0.29	-0.65 ± 0.31	0.01 ± 0.22	**0.32 ± 0.10**

Studies on ostrich behaviour and temperament

Ostriches are known for their erratic, unpredictable and sometimes cantankerous behaviour (Cloete and Malecki, 2011). Yet, the successful implementation of an artificial insemination programme in this species is believed to rely on harnessing the natural behaviour of ostriches (Malecki *et al.,* 2008; Malecki and Rybnik-Trzaskowska, 2011). Against this background, comprehensive studies have been undertaken to determine if positive interactions with humans at an early age would result in a change in their behaviour as juveniles (Bonato *et al*., 2013). It was established in the latter study that extensive human presence, including touch interactions, shortly after hatching and during the early rearing stages, resulted in juvenile birds more likely to allow touch interactions with humans and to approach human operators than birds subjected to standard care (i.e. the provision of food and water without further interactions) and those raised by foster parents, with almost no human interactions. Moreover, it was also found that these behavioural attributes were repeatable (Bonato *et al*., 2013). The advantages of extensive care on the body weight, survival and immune competence of chicks were recently confirmed by Muvhali *et al*. (2020a). Ostrich chicks exposed to extensive human presence (including touch at an early age) also had reduced physiological stress sensitivity later in life compared to chicks exposed to limited human presence and were able to distinguish between a familiar and an unfamiliar handler and adjust their behaviour accordingly (Muvhali *et al*., 2018). The advantages of extensive human care on the willingness of juvenile birds to interact with humans were recently confirmed by using a combined database of all previous studies (Muvhali, 2022). Moreover, the combined database also allowed the partitioning of between-animal variances in direct genetic and animal permanent environmental effects. These analyses confirmed that temperament traits of ostriches were heritable. Additional analyses also indicated that temperament was mostly genetically uncorrelated with slaughter and skin traits, with the exception of hair follicle score, where an unfavourable, yet unexplained, correlation was noted (Muvhali, 2022).

The case for artificial insemination in ostriches

So far, all genetic parameters for ostriches discussed have been primarily derived from pair mated ostriches stemming from the same resource population. This situation is far from optimal, because of the confounding of random effects, mate compatibility and the high number of males that need to be maintained per female (Cloete *et al.,* 2008b; Cloete and Malecki, 2011). Artificial insemination is therefore seen as a viable alternative to natural mating in this species (Malecki *et al*., 2008; Malecki and Rybnik-Trzaskowska, 2011). This technology has the potential to reduce the male to female ratio, while it also has the potential to contribute to occupational health and safety by reducing the number of aggressive males farmed with, as well as improving bird welfare, by reducing incompatibility between animals paired off in small paddocks under a pair-maiting system (Cloete and Malecki, 2011).

As voluntary (presumably stress-free) semen collection methods have since been developed by relying on the natural cooperation of trained ostrich males (Rybnik *et al*., 2007), there has been a number of distinct advances towards making artificial insemination a reality in this species. It has been demonstrated that semen can be collected from males twice daily by using by using the dummy female method, without compromising ejaculate output and sperm quality (Bonato *et al*., 2011). Moreover, further studies elucidated seasonal effects on the performance of male and female ostriches maintained for artificial insemination (Bonato *et* al., 2014; 2017; Muvhali *et al*., 2020b; 2022a). However, the question remains whether heritability and repeatability estimated from pair-bred ostriches would also be applicable to those used in an artificial insemination program.

Between-male differences in ejaculate traits were commonly reported (See Bonato *et al*., 2011; 2014; Muvhali *et al*., 2020b). These repeated records produced by the same male were therefore accommodated in two ways: firstly, the variance component of unique males across years was considered as an indication of the male permanent environment effects (pe^2) and secondly, the correspondence of records of unique males within years was considered as an indication of male temporary environment effects (te^2). The former parameter can be considered to reflect long-term effects of specific males on traits while te^2 rather reflect short term effects. It was not yet attempted to partition the pe^2 variance component in genetic and permanent environmental effects, given the relatively small number of males involved (28 in the latest study by Muvhali *et al*., 2022b). Long-term animal effects across years predominated in semen volume and libido scores in the study of Cloete *et al*. (2015). In contrast, short term effects within years seemed to be more important with regard to sperm concentration and morphology. The more recent study of Muvhali *et al*. (2022b) suggested pe^2 effects above 0.15 for mass sperm motility, sperm concentration and total number of sperm produced. Long-term pe^2 effects were mostly below 0.10 for computer-assisted sperm analysis traits, with the exception of the amplitude of the lateral head displacement (0.11) and wobble (0.14). Short-term te^2 effects were mostly below 0.10, except for beat cross frequency (0.10) and total number of sperm produced (0.11) (Muvhali *et al*., 2022b). Based on these results, some male specific semen traits seemed to be repeatable across production seasons, suggesting that these records can be used to select males with a high libido, good semen yield and/or morphologically sound spermatozoa for usage in the current flock. It is important to state, though, that further refinement is still needed as far as these male traits are concerned.

Repeated monthly records produced by the same female were similarly used to derive pe^2 and te^2 effects in a female flock selected to produce eggs in the absence of males (Bonato *et al*., 2017). Initial estimates suggested a pe^2 estimate of 0.21 for monthly egg production and 0.47 for mean egg weight (Cloete *et al*., 2015). Corresponding te^2 variance ratios were respectively 0.11 and 0.15. As more records accrued, thereby allowing the partitioning of female genetic and permanent environmental effects, Muvhali *et al*. (2022a) reported that these effects largely vied for the same variance as suggested by fairly similar log likelihood values when fitted separately. This study yielded h^2 estimates of 0.13 for monthly egg production and 0.40 for mean egg weight, with a genetic correlation of -0.20 between the traits. Corresponding te^2 effects amounted to respectively 0.10 and 0.19. The parameters reported were close to the pe^2 estimate of 0.17 (the sum of h^2, pe^2 and the service sire variance ratio) and the te^2 estimate of 0.15 derived by Fair *et al*. (2011) for monthly egg production records of pair-mated females. Corresponding estimates for egg weight amounted to 0.57 for pe^2 and 0.13 for te^2, which also corresponded fairly well with the present estimates. From these results it seems as if parameters derived for a flock of females producing eggs in the absence of males are quite similar to those derived from pair-mated females. It is also notable that a study involving insemination frequency and sperm dose in females maintained for artificial insemination reported moderate repeatability estimates of 0.44

for fertility rate and 0.40 for the fertile period after insemination (Muvhali *et al*., 2022c). If these estimates can be confirmed as more data accrue, it seems as if at least current flock selection of females for performance in an artificial insemination production system is feasible.

Genetic parameters across trait complexes

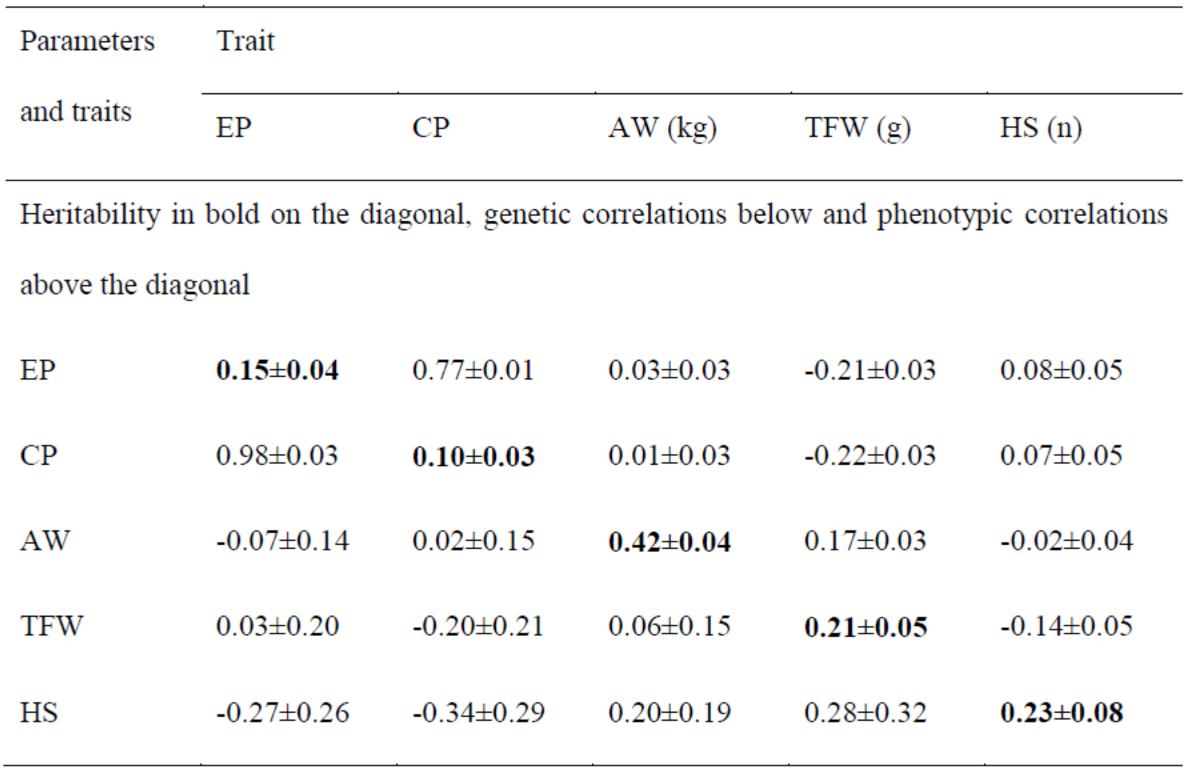
So far, most genetic parameters were derived within trait complexes. Genetic correlations were thus recently derived between traits measured repeatedly in mature birds (live weight, egg production and chick production) and slaughter traits (slaughter weight, skin size, nodule size score and hair follicle score) in their offspring (see Cloete *et al*., 2019). The genetic parameters within trait complexes were generally as in previous studies (see Cloete *et al*., 2008a; 2008b; Engelbrecht, 2013; Nemutandani, 2022). Adult weight was genetically positively correlated with slaughter weight, skin size and nodule size score (Table 4; Cloete et al., 2019). Reproduction traits of mature females were generally not correlated with offspring slaughter traits, barring an unfavourable genetic correlation of egg production with skin size. It is notable that the genetic correlations of reproduction with slaughter weight and skin size were consistently negative in direction, though.

In a study incorporating adult traits (egg production, chick production, adult weight, total feather weight, and adult hair score), all traits were heritable, estimates ranging from 0.10 for chick production to 0.42 for adult weight (Table 5; Cloete *et al*., 2022). The genetic correlation between egg and chick production that approached unity was the only significant genetic correlation. The absolute direction of the negative genetic correlations of reproduction with adult hair scores was favourable. The absolute direction of the genetic correlation of total feather weight with chick production was unfavourable, but of the same magnitude as the standard error.

Table 4. Genetic correlations of the traits in the slaughter animal complex with those in the mature animal complex (source: Cloete *et al*., 2019)

Mature animal traits	Slaughter traits			
	Slaughter weight (kg)	Skin size (dm^2)	Nodule size score (n)	Hair follicle score (n)
Egg production (n)	-0.15 ± 0.15	-0.41 ± 0.20	-0.10 ± 0.17	0.15 ± 0.17
Chick production (n)	-0.21 ± 0.18	-0.43 ± 0.24	0.05 ± 0.20	0.28 ± 0.18
Adult weight (kg)	0.81 ± 0.05	0.65 ± 0.12	0.31 ± 0.11	0.11 ± 0.11

Table 5. (Co)variance ratios (±SE) for and among egg production (EP), chick production (CP), adult weight (AW), total feather weight (TFW) and hair score (HS) in adult ostriches



Parameters and traits	Trait				
	EP	CP	AW (kg)	TFW (g)	HS (n)
Heritability in bold on the diagonal, genetic correlations below and phenotypic correlations above the diagonal					
EP	**0.15±0.04**	0.77±0.01	0.03±0.03	-0.21±0.03	0.08±0.05
CP	0.98±0.03	**0.10±0.03**	0.01±0.03	-0.22±0.03	0.07±0.05
AW	-0.07±0.14	0.02±0.15	**0.42±0.04**	0.17±0.03	-0.02±0.04
TFW	0.03±0.20	-0.20±0.21	0.06±0.15	**0.21±0.05**	-0.14±0.05
HS	-0.27±0.26	-0.34±0.29	0.20±0.19	0.28±0.32	**0.23±0.08**

Topics for further study

Unacceptable high levels of embryo and chick mortality remains a problem in the ostrich industry and further studies are needed to better understand the aetiology of these sources of reproductive failure. A recent study by Videvall *et al*. (2020) established that the microbiome in the gut of ostrich chicks that succumbed differed from survivors, although direct causative relations could not be established. This may be an avenue for further research involving both embryo and chick mortality. Despite the positive results in preliminary studies on traits important for artificial insemination, Muvhali (2022) listed several areas for further study as far as the further refinement of a workable protocol is concerned. Obvious combinations for estimating genetic correlations are reproduction and temperament, embryo and chick survival with reproduction as well as repeated feather traits of mature birds with traits in the growth and slaughter bird complex. Important trait complexes not yet studied are feather traits in juvenile birds and resistance to external stressors associated with commercial ostrich farming systems. A recent study on the response of breeder birds to climatic extremes may shape future approaches to dealing with selection of breeder birds for robustness in the species (Schou *et al*., 2022). Information on all these trait combinations is needed to ensure that ostrich breeding is conducted in an ethical and sustainable way. It will also be important to combine all traits of economic, sustainability and ethical importance in an overall selection index that will combine the aspirations of farmers, industry stakeholders and consumers alike. Such an index could build on previous efforts in this respect (Cloete *et al*., 2008b; Engelbrecht *et al*., 2019).

Concluding remarks and future outlook

This study reports notable research progress in the field of ostrich breeding since the last review. It is important, though, to realise that there are still various issues to be resolved, as mentioned under the previous heading. The multidisciplinary nature of many of these issues will need multi-institutional and multi-national teams to resolve. It is important to build local capacity to conduct these cutting-edge studies, but also to collaborate widely to make use of expertise that is not necessarily present within South Africa. If present project teams could be maintained and expanded to provide for additional needs as new techniques and technologies become available it would bode well for the ostrich industry. However, it is important to ensure that funding can be sourced to make this possible.

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Figure 1. Predicted means (±SE) depicting the effects of female age (2, 5, 6 and 9 years) and evaporative water loss on total embryo mortality in ostriches when either a second-degree polynomial (a) or a fixed linear regression and a random cubic spline approach (b) were used to model the relation of embryo losses to water loss (source: Z. Brand, unpublished)

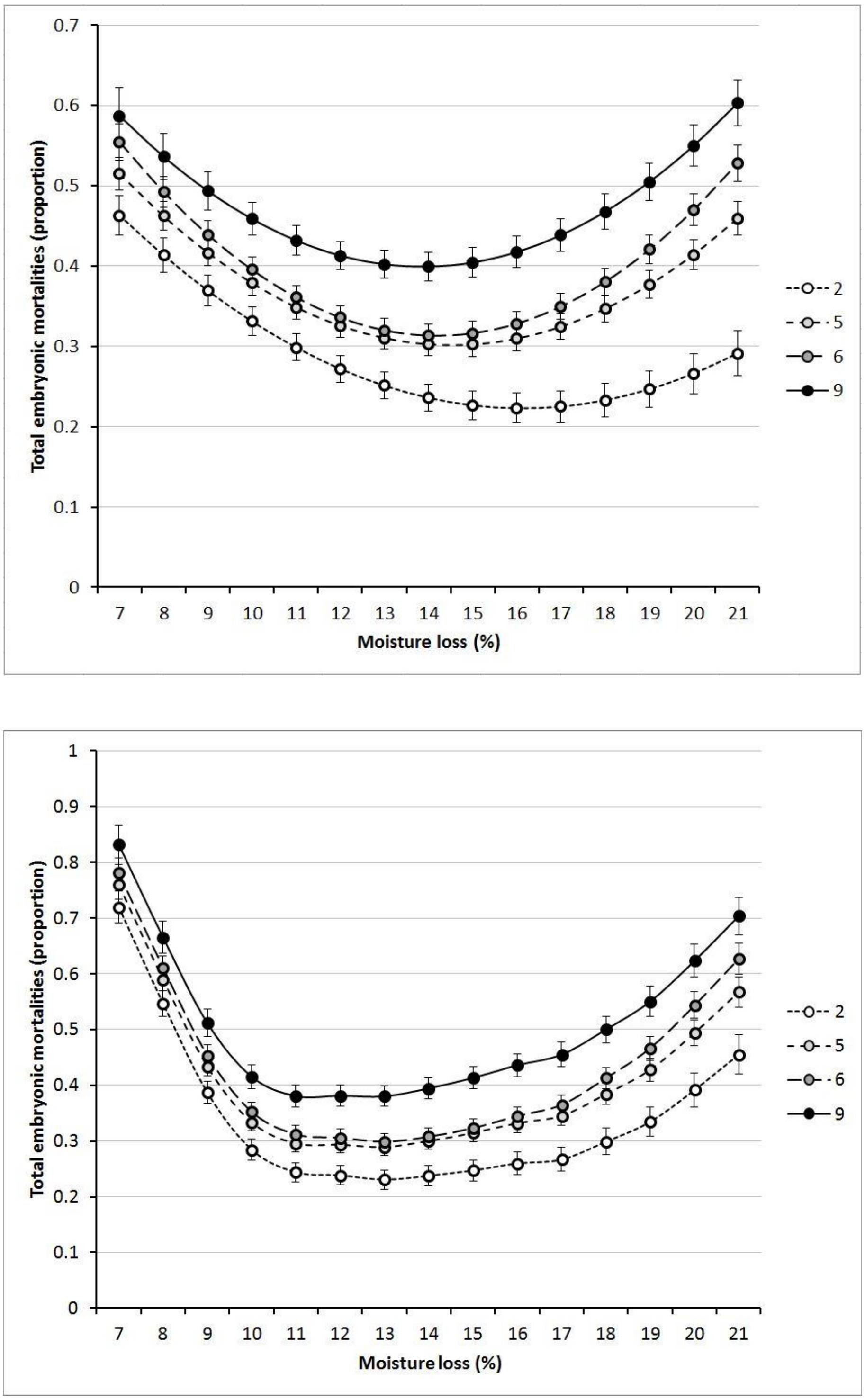
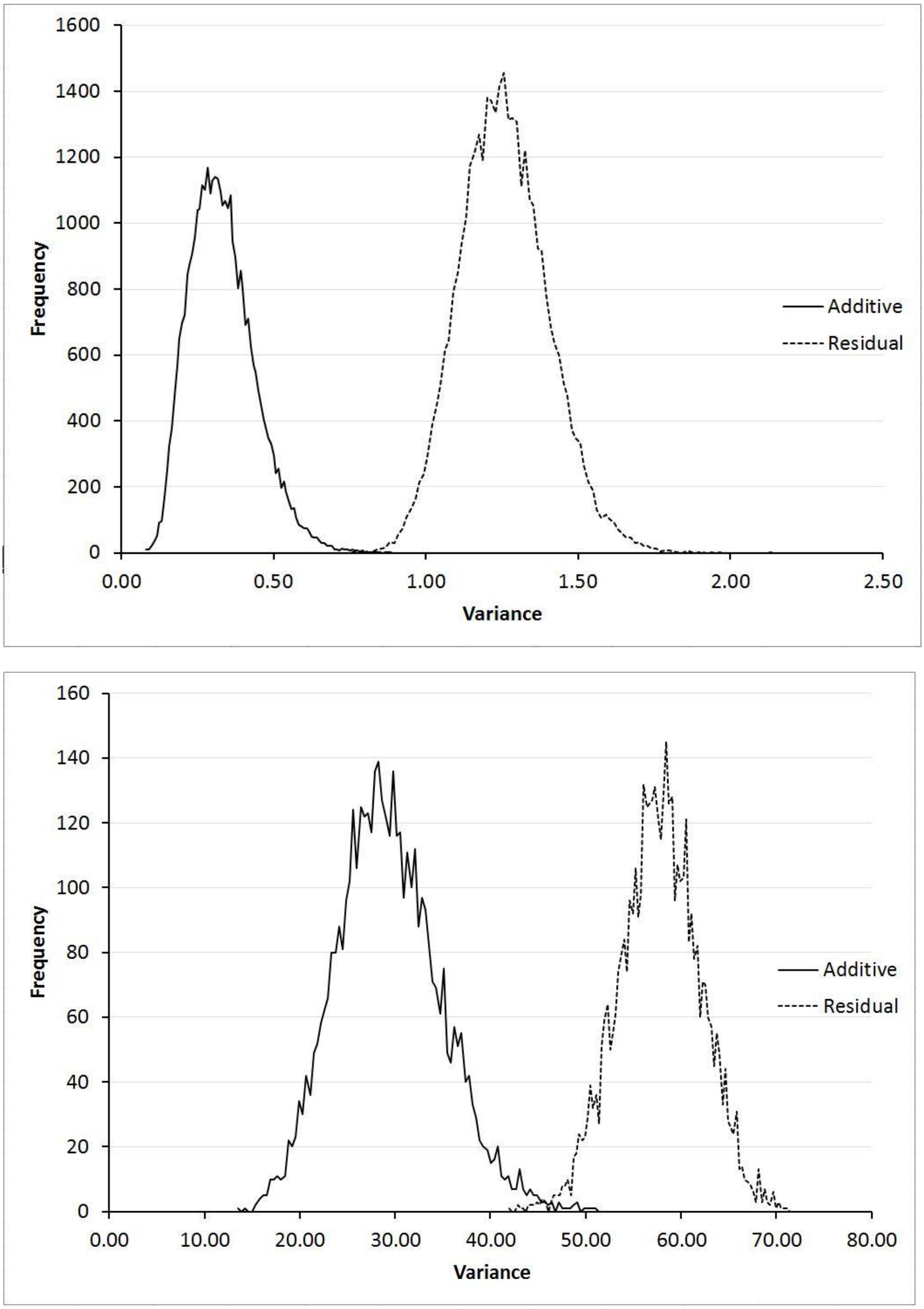


Figure 2. Posterior density distributions for the genetic (additive) and environmental (residual) variances pertaining to pitting score as a threshold trait (a) and the normally distributed trait skin size as a linear trait (b) (source: Nemutandani, 2022)



Construction of meat quality

Multidisciplinary approaches for reducing impacts of broiler meat quality defects (M. Petracci)

Massimiliano PETRACCI

M. Petracci, G. Baldi, M.A. Gagliano and F. Soglia
Department of Agricultural and Food Sciences, *Alma Mater Studiorum* - University of Bologna, Piazza Goidanich 60, 47521 Cesena, Italy
Corresponding author: m.petracci@unibo.it

Summary

Artificial selection for fast-growing and high-breast-yield hybrids has considerably marked up the pressure on breast muscle development, leading to the appearance and expansion of growth-related breast meat abnormalities myopathies (i.e. White Striping, Wooden Breast and Spaghetti Meat) affecting the pectoral muscle of heavy and fast-growing birds. Occurrence of these muscular abnormalities negatively impact both visual aspect and quality properties of raw and processed meat, causing relevant economic damages for the poultry industry. In the past few years, several studies have been carried out to investigate the biological and genetic mechanisms involved in their occurrence with special emphasis on White Striping and Wooden Breast conditions. Main features deal with hypoxia, oxidative stress, dysregulation of energy and carbohydrate metabolism, metabolic shift, vascular damage, and muscle development. It is commonly recognized that the occurrence of growth-related abnormalities boosts with increasing growth rate, slaughter age as well breast yield and weight. Within this context, it seems that artificial selection for broilers growth is close to biological limits and further improvements might be restrained by muscle biological potential and related animal welfare concerns. Thus, attempts have been made in the field of animal nutrition to reduce the occurrence of abnormalities through the modulation of both feed formulation (i.e. dietary supplementation of antioxidants, organic minerals, vitamins and aminoacids) or dietary intake through feed restriction. At slaughter plants, otherwise the most efficient solution seems the incorporation of downgraded meat into the formulation of finely and coarsely minced processed products. In this scenario, it has been also recently suggested that particular attention should be given on the modulation of embryonic formation of additional myofibers, instead of relying on post-hatch selection aimed at increasing muscle mass accretion. This review is therefore intended to make a summary of the possible causative mechanisms and forthcoming methods for mitigation of the most important qualitative issues affecting the chicken meat of fast-growing broilers.

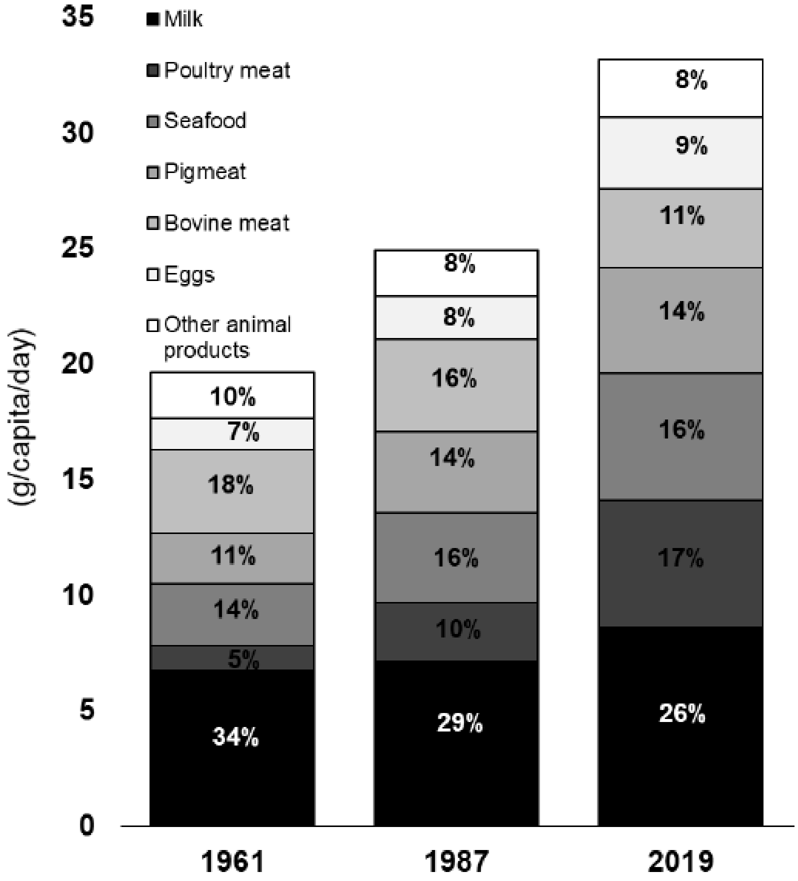
Keywords: Broiler, fast-growth, breast muscle hypertrophy, abnormalities, downgrading, underlying mechanisms, mitigation strategies.

Introduction

Nowadays global meat production derives from a very limited number of mammal and bird species as almost 90% is obtained by chickens (35.4%), pigs (32.6%) and cattle (20.4%) (FAO, 2021). Overall, this shows the gradual loss of biodiversity in the human diet which is increasingly based on a few animal and plant species (Lachat et al., 2018; FAO, 2019). Within this context, poultry production and consumption have increased substantially over the last decades and nowadays poultry meat is one of the primary animal protein sources for many people in several countries (Figure 1) (OECD/FAO, 2021). The universal success of chicken meat is found in its affordability, nutritional and sensory properties, ease of preparation as well as the absence of religious restraints (Baldi et al., 2020).

The development in industrialization and specialization of broiler meat production chains that took place starting from the end of World War II allows to obtain huge advancements in both the efficiency and the chicken meat production (Maharjan et al., 2021; NCC, 2022) (Table 1).

Figure 1. Evolution from 1961 to 2019 of the global animal protein supply quantity. Own design, data source: FAO (2022).



In addition, over the last decades, the lifestyle changes have also dramatically modified the way in which the poultry meat is marketed and consumed and therefore food technologies have become part of the poultry industry, and today much of the production is marketed in the form of cut-up and processed products (Baldi et al., 2020).

Indeed, due to the shift of consumers propensity to the convenience of ready-to-cook meals, nowadays almost half of the American meat market involves the commercialization of processed products (Table 1). Because of this market change, the genetic background of modern meat-type chickens has been profoundly adapted by increasing meat-yield and the proportion of high-value parts such as breast (Petracci et al., 2015; Tixier-Boichard, 2020) as shown in Table 2. While the large decline in days to market, coupled with the remarkable boost in breast size, has disclosed huge advancements in broiler productivity, it has also coincided with the development and expansion of muscular defects that affect the breast muscles of fast-growing broiler chickens.

Table 1. Progress of broiler performance and evolution of market segments and forms of chicken meat in the US (adapted from NCC, 2022).

	Live performances				Market segments		Market forms		
Year	market age (d)	market weight (kg)	feed to meat gain (kg)	mortality (%)	retail grocery (%)	food-service (%)	whole (%)	cut-up parts (%)	processed (%)
1940	85	1.30	4.0	12	-	-	-	-	-
1950	70	1.40	3.0	8	-	-	-	-	-
1960	63	1.52	2.5	6	-	-	78	19	3
1970	56	1.64	2.25	5	75	25	70	26	4
1980	53	1.78	2.05	5	71	29	50	40	10
1990	48	1.98	2.00	5	59	41	18	56	26
2000	47	2.28	1.95	5	58	42	10	44	46
2010	47	2.59	1.92	4	56	44	12	43	45
2020	47	2.91	1.79	5	55	45	9	40	50

Table 2. Progress in breast weight and yield in chicken strains used for meat production (data referred to male chickens).

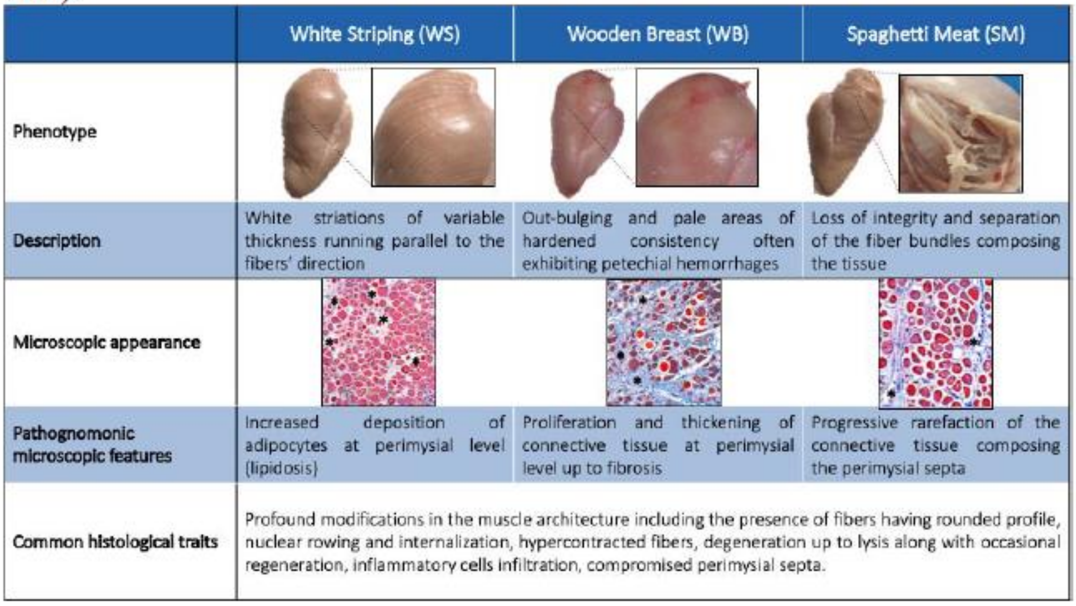
Year	Hybrid	Body weight (g)	Age (d)	Carcass yield (%)	Breast wt. (g)	Breast yield (%)
1957[1]	ACRBC	1,101	85	65.2	133	12.1
2001[1]	Ross 308	2,207	43	72.3	349	15.8
2007[2]	Ross 308	2,200	36	71.8	410	18.6
2012[2]	Ross 308	2,200	35	71.8	464	21.1
2017[2]	Ross 308	2,200	34	72.5	484	22.0
2019[2]	Ross 308	2,200	33	72.6	516	23.5

[1] Havenstein et al. (2003); [2] Ross 308 Broiler Performance Objectives

In the past decade, a new group of emerging muscular defects termed as White Striping (WS), Wooden Breast (WB) and Spaghetti Meat (SM) have raised the attention of the scientific community due to their noteworthy incidence levels along with the detrimental implications on meat quality and saleability. Albeit occurrence levels of affected meat might vary depending on country, animal age and weight at slaughter as well as classification criteria, it is assumed that these muscular defects appear in all countries where fast-growing strains are used for meat production (Petracci et al., 2019). Phenotypic and microscopic features of the Pectoralis major muscles affected by White Striping, Wooden Breast and Spaghetti Meat conditions are reported in Figure 2. White striping was first noted in 2009 (Kuttapan et al., 2009) and wooden breast myopathy in

2013 (Sihvo et al., 2014), while Spaghetti Meat defect was first recounted in 2015 with the name of “Mushy Breast” by Bilgili (2015). Albeit their distinctive phenotypes, WS, WB and SM conditions entail common histological features, thus suggesting that they might share at least some common causative mechanism that triggers their occurrence (Figure 2).

Figure 2. Phenotype and microscopic traits of broilers’ Pectoralis major muscles affected by white striping, wooden breast, and spaghetti meat abnormality (Adapted from Soglia et al., 2021).



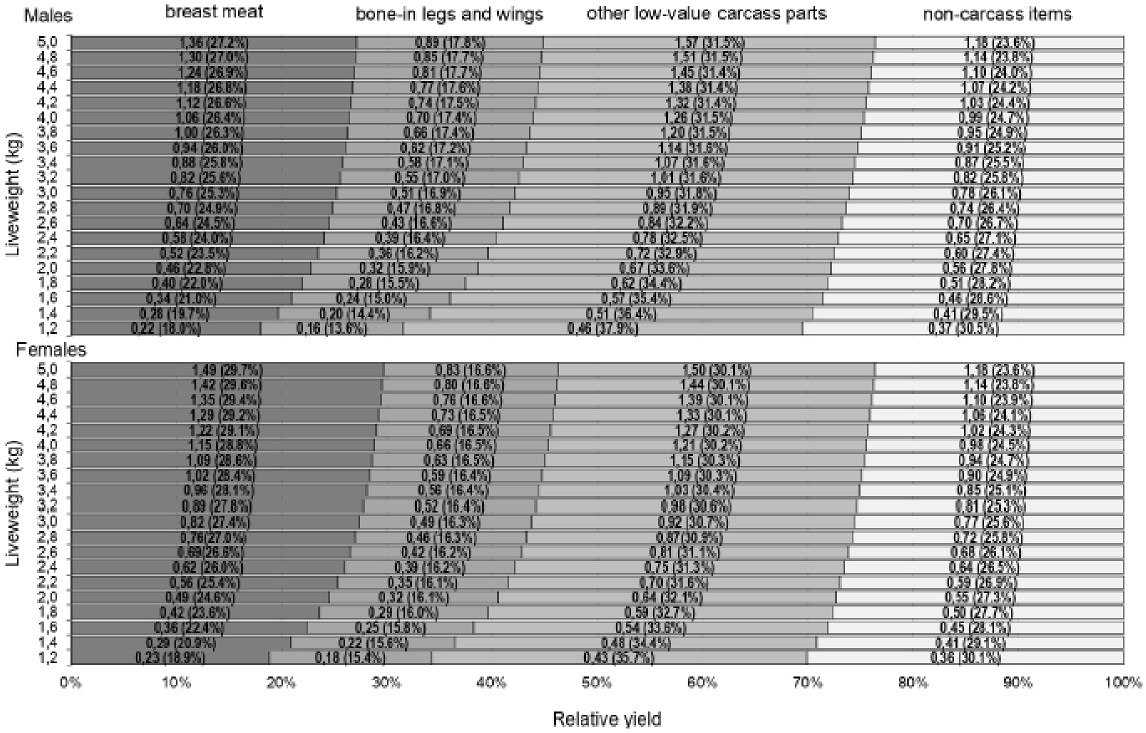
	White Striping (WS)	Wooden Breast (WB)	Spaghetti Meat (SM)
Phenotype			
Description	White striations of variable thickness running parallel to the fibers’ direction	Out-bulging and pale areas of hardened consistency often exhibiting petechial hemorrhages	Loss of integrity and separation of the fiber bundles composing the tissue
Microscopic appearance			
Pathognomonic microscopic features	Increased deposition of adipocytes at perimysial level (lipidosis)	Proliferation and thickening of connective tissue at perimysial level up to fibrosis	Progressive rarefaction of the connective tissue composing the perimysial septa
Common histological traits	Profound modifications in the muscle architecture including the presence of fibers having rounded profile, nuclear rowing and internalization, hypercontracted fibers, degeneration up to lysis along with occasional regeneration, inflammatory cells infiltration, compromised perimysial septa.		

Nowadays, it is estimated that deboned breast and leg meat (without skin and bone) may reach up to 47% of live weight in birds slaughtered at weigh higher than 3 kg (Aviagen, 2021) (Figure 3).

Origin of growth-related abnormalities

In the past few years, several studies have been carried out with the aim of identifying the causative mechanisms and triggering factors responsible for the onset of the growth-related abnormalities (i.e., WS, WB, SM) and underlying the subsequent network of events leading to their development (Mutryn et al., 2015; Velleman and Clark, 2015; Alnahhas et al., 2016; Zambonelli et al., 2016; Papah et al., 2018; Pampouille et al., 2018, 2019; Brothers et al., 2019; Marchesi et al., 2019; Papah and Abasht, 2019; Soglia et al., 2020; Bordini et al., 2021). However, despite the remarkable knowledge gained concerning the microscopic features and the gene expression profile of the affected muscles, the primary cause initiating the process is not clear so far. Indeed, the outcomes of the studies performed at genetic level did not reveal the existence of a major causative gene but rather support a polygenic inheritance of these defects (Pampouille et al., 2018).

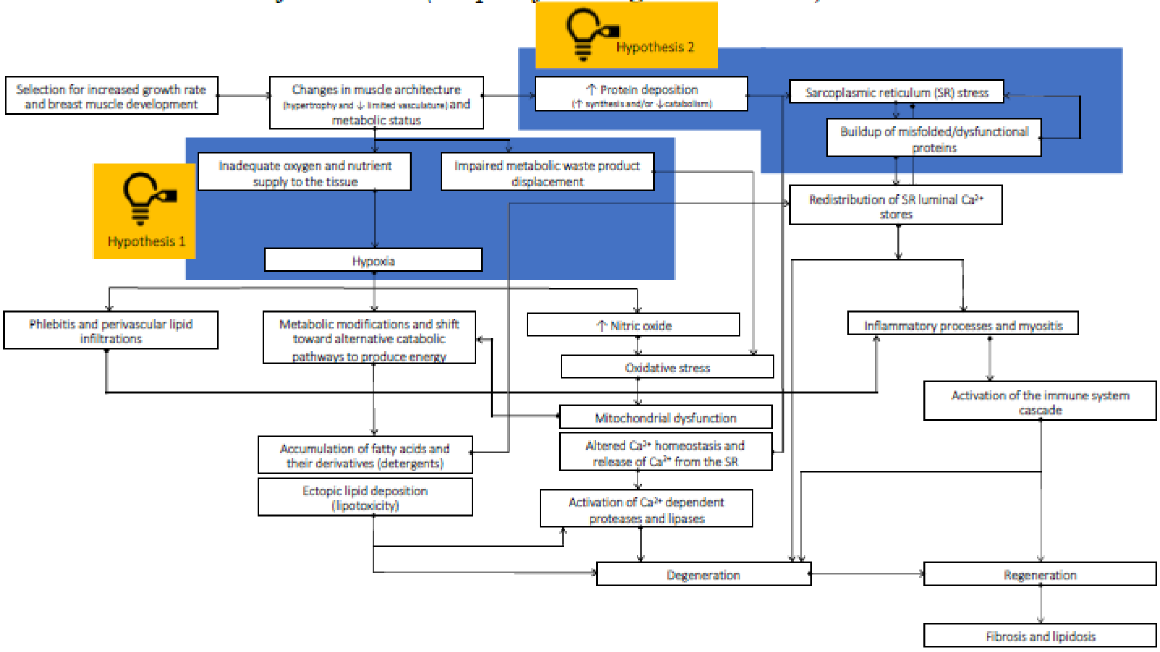
Figure 3. Body composition of male and female chickens as affected by slaughter weight in Ross 308 hybrid. Own design, data source: Aviagen, 2021.



Examinations performed at histological level evidenced peculiar features associated with the occurrence of these disorders including an abnormal deposition of adipose tissue (WS), a proliferation and thickening and a progressive rarefaction of the connective tissue (in WB and SM, respectively) at perimysial level (Figure 2). These distinctive histological traits partly account for the phenotypes of the affected muscles which may also profoundly differ. Besides that, the evidence that WS, WB, and SM affected muscles share common microscopic traits seems to support the hypothesis of the existence of a common causative network of events underlying their occurrence (Soglia et al., 2021).

Within this context, a low vascularization of the *Pectoralis major* muscle (due to its hypertrophic growth) is currently considered as the most feasible phenomenon that, leading to hypoxia, likely triggers the development of these disorders (Mutryn et al., 2015; Sihvo et al., 2017; Marchesi et al., 2019; Abasht et al., 2019; Malila et al., 2019; Pampouille et al., 2019; Soglia et al., 2021). Recent findings, further corroborating this hypothesis (Bordini et al., 2021), built a basis for supposing a key role of endoplasmic reticulum stress, responsible for protein folding, in the development of these defects. Indeed, the increase in protein synthesis which is required to support the hypertrophic growth of the pectoral muscle potentially overburdens the capacity of the sarcoplasmic reticulum thus leading to the accumulation of misfolded and/or dysfunctional proteins. This hypothesis is supported by the evidence that alterations in this cellular compartment along with a subsequent activation of the Unfolded Protein Response are among the first ultrastructural and molecular changes associated to an early onset of WB (Papah et al., 2018; Sihvo et al., 2018).

Figure 4. Schematic representation of the possible time series and network of events involved in the onset and triggering the occurrence of the growth-related abnormalities affecting broilers' Pectoralis major muscles (adapted from Soglia et al., 2021).



Then, once that the process has been established, a time-series sequence of events (i.e., phlebitis, oxidative stress, altered calcium homeostasis, etc.) is initiated thus resulting in the activation of complex response mechanisms (i.e., modifications in the energetic metabolism, inflammation, degeneration, and regeneration) (summarized in Figure 4) which ultimately led to the development of the growth-related defects. An extensive description of the network of events likely resulting in the development of WS, WB, and SM abnormalities can be found in the review paper published by Soglia et al. (2021).

Attempts for mitigation

Within this scenario, solutions for avoiding and/or mitigating the occurrence of muscular abnormalities are drawing the attention of the scientific community. The incidence of muscular defects increases with increasing growth rate, slaughter age and weight (Lorenzi et al., 2014; Papah et al., 2017), however Radaelli et al. (2017) reported that the first signs of muscle fiber degeneration associated to muscular abnormalities in fast-growing chickens are visible already at 14 days of age. Thus, attempts in the field of animal nutrition have been made with the purpose of reducing both the severity and the occurrence of WS, WB and SM by the modulation of dietary intake (i.e. feed restriction) or feed formulation (e.g. supplementation of antioxidants, organic minerals, aminoacids, vitamins etc.) (Sirri et al., 2016 ; Livingston et al., 2018 ; Zampiga et al., 2018 ; Bodle et al., 2018 ; Meloche et al., 2018 ; Lackner et al., 2022). However, these strategies might not result in any effective mitigation effect because a possible reduction of the incidence of breast abnormalities might be imputable to a decreased slaughter weight and breast size of the animals (Petracci et al., 2019). Thus, incorporating abnormal meat into the formulation of processed products seems the most practical solution, since mincing procedures as well as the addition of functional ingredients might partially conceal the impaired sensory and technological properties of abnormal meat (Carvalho et al., 2021; Santos et al., 2021). Furthermore, since muscular abnormalities mainly affect the superficial section of breast muscles, addressing it for the manufacture of processed products and breast fillet's deep section for fresh retailing could be a strategy to limit the amount of downgraded meat (Baldi et al., 2019). Currently, the interest of scientific research has been also focused on the application of physical (i.e., ultrasounds, pulsed electric field, high pressure processing) and/or chemical (i.e., marinating, enzymes) procedures that might be helpful to improve technological and sensory properties of abnormal meat during processing (Starcevic et al., 2021). A relevant matter for the broiler industry is also the early detection and objective grading of meat affected by muscular abnormalities through reliable and non-destructive methods, which may prevent the need to hire and train on-line personnel. Traffano-Schiffo et al. (2017) proposed radiofrequency spectra as an effective technique to detect WS in chicken carcasses with skin, while hyperspectral imaging (i.e. a novel technique that combines spectroscopy with imaging) was successfully applied to discriminate between normal and WS breast muscles by simultaneously providing information related to chemical and physical characteristics of meat (Jiang et al., 2019). Moreover, NIR spectroscopy lines (Geronimo et al., 2019) and a sideview imaging system (Yoon et al., 2022) have been also used to efficiently detect WB meat in chicken slaughtering lines.

Conclusions

Despite all the efforts made by the scientific community during the past decade, no efficient solutions capable to inhibiting the onset of muscular abnormalities or at least lessening the symptoms and consequences on the quality of the meat have been elucidated. Taking a step back seems unavoidable by now: further pressure exerted on breast muscle development might be restrained by muscle biological potential. In this scenario, albeit solving the issue at its roots appears complex so far, the meat industry will need to re-evaluate selection strategies and opt for more sustainable solutions. In addition, further scientific researches should be addressed on embryonic formation of additional myofibers, feeding strategies as well as innovative processing solutions aimed at reducing both the economic- and meat quality-related impact of growth-related abnormalities.

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Influence of digestive kinetics on broiler performance (A. Jansman)

Alfons JANSMAN

Wageningen Livestock Research,
P.O. Box 338, 6700 AH Wageningen, The Netherlands
Corresponding author: alfons.jansman@wur.nl

In current feed evaluation systems, the nutritional value of ingredients in poultry diets is based on determination of ileal (proteins and amino acids) and fecal (starch, sugars, fat and minerals) nutrient digestibility values. However, not only the extent but also digestive kinetics can largely vary between ingredients, in particular for starch, protein and amino acids. Differences in digestion kinetics mainly relate to variation among ingredients in physical and chemical structure in which the nutrients are kept. In turn, these determine nutrient solubility, passage rate, hydrolysis and absorption rate in the different compartments of the digestive tract and variation in time between nutrient ingestion and availability of nutrients at organ and tissue level for synthesis and metabolism purposes. Many synthesis processes require simultaneous availability of different nutrients (e.g. amino acids and energy (ATP)). Asynchrony of nutrient availability might result in a less efficient utilization of nutrients and finally affect performance. Broilers fed diets with more slowly digestible starch showed to increase body weight gain and improve feed conversion ratio. In addition, body protein retention was found to be negatively correlated to the starch digestion rate in broilers fed sorghum-based diets, indicating that slowly digestible starch can increase body protein retention in broilers. Thus, the fate of dietary amino acids in the post absorptive metabolism depends on the kinetics of protein digestion relative to the digestion kinetics of energy providing nutrients in the diet such as starch. More focus on nutrient digestion kinetics during formulation of poultry diets could increase nutrient efficiency and sustainability in poultry production.

Construction of egg quality

Recent advances on the involvement of extracellular vesicles during avian eggshell formation (J. Gautron)

Joël GAUTRON

BOA, INRAE, Université de Tours, 37380 Nouzilly, France

Corresponding author: Joel.gautron@inrae.fr

Abbreviated title: Review on avian eggshell biomineralization

Summary

The eggshell is a critical barrier against mechanical stresses and microbial penetration. Its integrity is essential to maintain the hygienic quality of this basic human food and to limit the number of downgraded eggs. The eggshell is made of 95% mineral phase (calcium carbonate on calcite form) and an organic matrix (3.5%) mostly containing proteins. Eggshell formation arises from an extra-cellular biomineralization process. We describe in this review, the latest advances in the formation of the eggshell, which takes place in a fluid that contains eggshell precursors and involves a transient phase of amorphous calcium carbonate (ACC). We also describe recent insight on the identification of transient amorphous calcium carbonate explaining this rapid mineralization process. We also report on the advances on the function of shell matrix proteins to interact with mineral, thus determining the crystal polymorph, the first event of nucleation and the final texture of the shell and consequently its resulting mechanical properties. The role of vesicular transport to provide stabilized ACC in chicken uterine fluid where mineralization takes place was also demonstrated recently. These extra-cellular vesicles play a crucial role in eggshell mineralization, in which annexins transfer calcium into vesicles and carbonic anhydrase 4 catalyzes the formation of HCO_3^-, for accumulation of ACC in vesicles. ACC is stabilized by ovalbumin and/or lysozyme or additional proteins identified in vesicles in this study. Finally, EDIL3 and MFGE8 are proposed to guide EVs to the mineralization site.

Keywords: Chicken, eggshell, biomineralization, calcium supply, extracellular vesicles

Introduction

The eggshell constitutes the external envelope of the eggs and fulfils five essential functions to allow the harmonious development of a chicken embryo. It prevents the dehydration of the internal environment of the terrestrial egg, it ensures a role of physical protection against the shocks, a thermic protection, it allows gas exchanges and it prevents the penetration of the microbes. The shell is the only non-consumable part of an egg, and a large number of socio-economic issues for the consumer egg industry will depend on its integrity and quality. Thus, cracked shells will lead to an economic loss for the producer and to food infection risks for the consumers. Moreover, in the current context of evolution of the societal demand for rearing systems with outdoor runs and an extension of the production period, the maintenance of the integrity of the shell is then preponderant to guarantee a healthy egg and preserving good mechanical properties (Gautron et al., 2021). The shell quality depends of numerous factors as genetics of the birds, the hen’s physiology, the environment, the nutrition and management of hens. Then finally the egg quality is depending of the « insult » that occur in the rearing system, the egg transport and egg sorting. Many of these factors impacting shell quality are perfectly controlled. The use of appropriate genetic, optimal nutrition, limit but do not eliminate the breakage, notably for elderly birds for which the egg percentage breakage can increase to 10-12% at the end of laying period.

Further improvement of the mechanical properties of the shell will be achieved by taking into account not only the mass of the shell, but also mechanisms largely dependent on the ultrastructure of the shell, i.e. the arrangement, shape and orientation of the constituent crystals that give the shell its structure and mechanical properties (Gautron et al., 2021, Nys et al., 2022). This manufacturing process is the result of an interaction between minerals and proteins secreted in the formation environment that control this process. The knowledge of these processes is crucial to allow the integration of this component in new genomic selection programs and also to study nutritional factors such as vitamin D whose metabolism could be limiting at different periods of the hen's life and, which could potentially be corrected by nutrition. The objective of this paper is therefore to review the state of knowledge on the mechanisms of eggshell biomineralization, in order to identify avenues for further improvement.

Eggshell structure, formation and composition

The chicken eggshell contains 1.6 % water, 3.3 to 3.5 % organic matrix when eggshell membranes are included and 95 % inorganic minerals. It is mainly made of calcium carbonate (98.4 % of its mineral part), which is pervaded by an organic matrix corresponding to 2.3 % of the shell weight. From inside to outside, six different layers are observed in the eggshell (Gautron et al., 2021). In chicken, the most documented bird, the eggshell is about 0.4 mm thick. The innermost layers are made of two shell membranes composed of interlacing protein fibers. There are two of them and they are entirely made up of organic matter. The inner shell membrane is 20 µm thick and is in contact with the egg white. It is from the outer shell membrane (50 µm) that the mineralisation of the shell is initiated to give rise to the mammillary layer. The mamillary layer of about 70 µm is the innermost part of the calcified layer. Its base consists of the mamillary knobs which are organic clusters deposited on the surface of the outer shell membrane and from which mineralisation is initiated. The mineralisation continues outwards, initially forming a cone or mamelon-like structure. The palisade layer begins when the multidirectional growth of the cones of the mamillary layer leads to a fusion of adjacent cones. The palisade layer is therefore a compact layer of minerals associated with an organic framework. This continuity is broken at the level of the pores which cross the shell from one side to the other to allow the gas exchanges necessary for the development of the embryo. A surface layer of small adjacent single calcite crystals is then deposited vertically on the surface of the palisade layer under the cuticle. The cuticle is the outermost layer of the egg and consists of organic material. The cuticle closes the pores and thus prevents the penetration of bacteria into the egg. Gas exchange is made possible by cracks that appear in the dried cuticle.

Shell mineralisation occurs in the uterine part of the oviduct of birds. When the egg enters the uterus five hours after ovulation of the yolk, it is a soft egg on which mineralisation will start in a process that will last about 17 hours in the laying hen. This process takes place in the lumen of the organ, where the physico-chemical conditions necessary for cell-free biomineralisation are present. Shell formation is temporally controlled, and in chickens four main steps can be identified during the 17 h process (from 5 h to 22 h post-ovulation) (Rodriguez-Navarro et al. 2015). They corresponded to the initial stages dominated by amorphous calcium carbonate (ACC) deposition on eggshell membranes (5 h p.o.), its progressive transformation to form calcite aggregates on mammillary knobs surrounded by ACC particle and the growth of large calcite units surrounded by ACC. Calcite crystals rapidly grow to form larger crystal units. The interaction with eggshell organic matrix components inhibits calcite crystal faces parallel to the c-axis, thus causing elongated crystal growth in this direction. Calcite crystals growing with their c-axis nearly perpendicular to the surface block the growth of adjacent crystals with less favourable orientations, resulting in the development of columnar calcite units. Finally, mineralization is terminated and a thin proteinaceous layer (cuticle) is deposited on the shell surface.

Molecular control of the avian eggshell biomineralization

There are two physiological processes that allow the mineralisation of the shell. They are the transfer mechanisms of the large quantity of minerals necessary for the formation of the shell and the biomineralisation process controlled by the organic matrix to give an ordered structure with exceptional mechanical properties.

Role of organic matrix proteins during eggshell biomineralization

During its formation, the shell is bathed in a uterine fluid (UF) secreted by uterine cells that contains the organic and mineral precursors necessary for shell calcification (Gautron et al., 1997). The transition of ions to a crystalline state is achieved through amorphous transitional forms allowing crystallisation under physiological conditions. In birds, calcium carbonate is initially deposited as an amorphous calcium carbonate phase (ACC), which progressively transforms into calcite (Rodriguez et al., 2015). Matrix proteins play a crucial role in this process. They stabilize ACC, promote crystal nucleation, select the calcite polymorph, and regulate the evolution of crystal size and morphology (Gautron et al., 2021; Dominiguez-Vera et al., 2000; Hernandez-Hernandez et al., 2008). These matrix–mineral interactions determine the orientation of calcite crystals, which results in the complex ultrastructure of the eggshell, its texture, and consequently, its mechanical properties. These observations have largely stimulated research to identify organic matrix proteins by proteomic and transcriptomic approaches. The set of sequences identified were grouped into 904, 697, 622, 475, 484 and 149 unique proteins constituting the chicken, turkey, quail, zebra finch, duck and Guinea fowl eggshell proteomes (Gautron et al., 2019; Mann and Mann, 2013; 2015; Mann, 2015; Le Roy et al., 2019). The role and function of these proteins in shell calcification has only been studied in chicken and only for a limited number (Gautron et al., 2021; Hincke et al., 2012). Among this large list of shell matrix proteins, are proteins with an established role in the biomineralization, which directly interact with the mineral phase to stabilize ACC and/or to modify the morphology of crystals that determine the eggshell ultrastructure of avian eggshells and their resulting mechanical properties. Another group is composed of proteins involved in the regulation of proteins directing mineralization. This group is made of uterine fluid proteins that interact with proteins directing mineralization. Indeed, mineralization takes place in an acellular medium and the proteins belonging to this group inhibit or activate proteins of the mineralization milieu. Some of these proteins may be involved in proper folding of eggshell matrix proteins to ensure an appropriate template for calcium and mineral interactions. Protease and protease inhibitors are also belonging to this group. They are believed to play specific and controlled roles during the calcification process, either by degrading proteins or regulating processing of proteins into their mature forms.

Regulation of calcium supply

The calcium metabolism linked to egg formation in birds is strongly exaggerated. Indeed, there is no calcium storage in the shell gland (uterus) before shell formation (Nys et al., 2022). Calcium is directly provided by ionic blood calcium, to supply daily the necessary 2 g of shell calcium. Calcium is provided by the hen diet, directly by intestinal absorption, although 40% of this is derived from bone mobilisation because of desynchronization between the period of feed intake (daytime) and shell formation, which mainly takes place during the night (Nys et Le Roy, 2018: Nys et al., 2022). Both components of the shell mineral (Ca_2^+ and CO_3^{2-}) are continuously supplied during eggshell formation via the blood plasma, firstly by trans-epithelial ionic transport through the uterine epithelium and secondly, by vesicular secretion of ACC mineral particles.

A comprehensive and further refined model for calcium and carbonate transport to the mineralization site during eggshell formation was recently proposed (Nys et al., 2018; Nys et al., 2022; Gautron et al., 2021). Calcium and carbon dioxide originate from the blood. Blood CO^2 passively diffuses into uterine cells (Hodges and Lörcher, 1967), where it is hydrated by Carbonic Anhydrase 2 (CA2). Alternatively, bicarbonate can be actively transferred into uterine cells using the Na^+/HCO_3^- co-transporters SLC4A4-A5-A10 (Nys and Le Roy, 2018). Bicarbonates are actively extruded from cells by the

HCO_3^-/Cl^- exchanger SLC26A9 (Nys and Le Roy, 2018). Additionally, bicarbonate ions can be directly produced in the uterine fluid by hydration of CO^2 by membrane-bound CA4, which has its active site in the extracellular space (Zhu et al., 1990). The transcellular pathway to secrete calcium and bicarbonate ions into the fluid has been previously described (Jonchère et al., 2012; Brionne et al., 2014). Plasma Ca^{2+} is transferred into uterine cells by transient receptor potential cation channels (TRPVs) and/or otopetrin 2 (OTOP2) and/or ATPase secretory pathway Ca2+ transporting 2 (ATP2C2) (Sah et al., 2018; Nys and Le Roy, 2018). Intracellular calcium ions are buffered/transferred by calbindin. Other Ca^{2+} pumps associated with the endoplasmic reticulum could also be involved in this transfer (ATP2A2/3 and ITPR1/2/3). Finally, the Ca_2^+/Na_2^+ exchangers SLC8A1-3 and the Ca_2^+ pumps ATP2B1-B2 are involved in the apical extrusion of calcium into the uterine fluid (Sah et al., 2018; Nys and Le Roy, 2018). Uterine Ca_2^+ secretion is quantitatively associated with calbindin levels and the regulation of uterine calcium transfer in conjunction with its synthesis has been studied in detail (Nys and Le Roy, 2018; Bar, 2009).

A paracellular Ca2+ uptake pathway is present in intestine and acts to replenish calcium from dietary sources during eggshell biomineralization when soluble calcium in the intestinal lumen creates a favorable gradient for passive absorption This intestinal paracellular pathway involves claudins (CLDN), occludins (OCN), junctional adhesion molecules (JAM) and tight junction proteins (TJP) (Gloux et al., 2019). RNA-Seq analysis reveals the expression of several genes of this paracellular pathway (Tjp1, Cldn1, Cldn10, Ocln, Jam2) (Gautron et al., 2020). Moreover, expression of Cldn10 has also been detected in chicken uterus (Sah et al., 2018; Yin et al., 2019). This paracellular pathway is probably contributing to the secretion of water and ions for osmotic regulation (K, Na) during the process of eggshell formation. The ionic calcium concentration in uterine fluid ranges from 6 to 10 mM depending of the stage of calcification (Nys et al., 1991), which is 6 times higher than blood calcium levels (1-2 mM); consequently, the concentration gradient is not in favor of calcium movement towards the uterine fluid through the paracellular pathway (Nys and Le Roy, 2018). However, Bar (2009) suggested that the electrical potential difference could invert this gradient, allowing some paracellular transfer of calcium into the uterine fluid. Consequently, the paracellular pathway could participate to maintain ionic and osmotic homeostasis.

Extracellular vesicles to transport and stabilize transient forms of calcium

More recently, Stapane et al (2019-2020), have demonstrated a vesicular mechanism to stabilise the transient forms of calcium carbonate necessary for calcite crystal formation. Evaluation of CaCO3 vesicular transport in chicken uterus was initiated following the observation of high levels of vesicular protein markers (EDIL3 and MFGE8) in eggshell and in uterine fluid during shell formation (Marie et al., 2015a). Bioinformatics tools, mRNA levels and protein quantification were used to explore the role of EDIL3 and MFGE8 in chicken eggshell biomineralization. In avian uterus, transmission electron microscopy (TEM) observations demonstrated the presence of intracellular vesicles (100 to 500 nm) in the cytoplasm of the epithelial ciliated cells (Stapane et al., 2020). Vesicles accumulate at the apical plasma membrane and bud to secrete extracellular vesicles (EVs), which were revealed in uterine fluid adjacent to the apical region of uterine cells (Stapane et al., 2020). The presence of calcium carbonate as ACC in the vesicles was confirmed by electron energy loss spectroscopy (EELS) and by energy-dispersive X-ray spectroscopic (EDS). Electron diffraction on EVs extracted from uterine fluid indicated that the calcium carbonate inside vesicles was amorphous, similar to the ACC previously identified at the initial stage of eggshell formation (Rodriguez-Navarro et al., 2015). This observation was further explored by studying the presence of major EV proteins using transcriptomics, proteomics and immunochemistry to decipher the origin and mechanisms of vesicle formation and function.

EDIL3 and MFGE8 bind to EVs budding from uterine cells into the uterine fluid, in order to guide vesicular transport of stabilized ACC for delivery to the mineralizing site and moreover prevent non-specific precipitation. Three annexins (Anxa 1, 2 and 8) are expressed at high levels in the uterus at the onset of shell formation (Stapane et al., 2020), in agreement with previous proteomics studies (Mann, 2006; Jonchere et al., 2012; Marie et al., 2015b), and were revealed in the epithelium (Anxa 1, 8) and tubular glands (Anxa 8) by immunochemistry. Annexins are Ca channels proposed to contribute to uptake of Ca for intra-vesicular ACC formation. EDIL3 is overexpressed in the uterus and is specific to the uterine fluid EV fraction (Stapane et al. 2019; 2020). This protein possesses an EGF-like calcium- binding domain and is hypothesized to guide EVs to the mineralisation front. Carbonic anhydrase 4 (CA4) is present in the epithelial cells and in EVs and is highly expressed at the early stage of shell formation. CA4 catalyzes the reversible hydration of CO2 forming HCO3 and might contribute to accumulation of ACC in vesicles.

A global representation of vesicular transport and molecular actors during eggshell mineralization was proposed (Stapane et al., 2020; Gautron et al., 2021; Nys et al., 2022). Annexins would promote calcium entry into EVs, whereas CA4 would catalyze the hydration of CO2 into bicarbonate ions. ACC accumulates inside EVs and is stabilized by specific proteins. EDIL3 and potentially MFGE8 would serve as guidance molecules to deliver vesicular ACC to the mineralization site. The quantitative contribution of the vesicular secretion of CaCO3, relative to the secretion of each ion by the transcellular pathway, remains to be explored.

Vitamin D and Regulation of the molecular actors involved in the shell calcification

If the calcium contained in the eggshell comes entirely from the food, there is a desynchronization between the need for calcium for the formation of the eggshell during the night and the dietary intake of this calcium during the day. To do this, the hen has a particular bone structure, the medullary bone, which is mobilized during the night to provide part of the calcium necessary for the calcification of the shell. During the day, when the hen has access to its food, the medullary part of the bone will be mineralized again (Nys et al., 2022). The regulation of calcium metabolism during shell formation in the hen involves many organs. First, the gut, which will allow the transfer of calcium to the bone and uterus, via the bloodstream. It also involves the uterus, which will have to transfer to the calcification site (uterine fluid), large quantities of calcium necessary for the formation of the shell while maintaining cellular homeostasis. Vitamin D and in particular its active metabolite ($1.25(OH)_2D_3$), will play a crucial role in the regulation of calcium transfers at the intestinal and bone level. Vitamin D is first hydroxylated to 25-hydroxycholecalciferol ($25\text{-}OH\text{-}D_3$) in the liver before being hydroxylated to $1.25(OH)_2D_3$ in the kidney (Christakos. et al, 2010). The use of the hydroxylated form ($25\text{-}OH\text{-}D_3$) in the feed has a metabolic advantage by avoiding the initial hepatic step and would allow for better availability of the intermediate vitamin D metabolite. This role at the uterine level has been little explored and it is generally accepted that vitamin D would have no effect at the uterine level in the hen. In a recent study (Gautron et al., 2022), hens were fed with vitamin D_3 and hydroxylated form ($25\text{-}OH\text{-}D_3$) and the expression level in the uterus was analyzed for 91 genes. Of these, 17 genes encode organic matrix proteins known to play a major role in shell mineralization and 65 encode transporters of calcium, bicarbonate and other ions necessary for mineralization. Additionally, 21 overexpressed genes code for paracellular transport proteins and 44 allow transcellular transfers. It is particularly notable that all of these genes are stimulated by $25\text{-}OH\text{-}D_3$. This study clearly shown that vitamin D plays an important role in the regulation of calcium and mineral transfers in the hen's uterus. This role is not limited to calcium transfers to the gut and bone as previously described. Furthermore, this study shows that the use of the hydroxylated form of vitamin D3 as $25\text{-}OH\text{-}D_3$ allows an overexpression of many genes involved in the transcellular, vesicular and paracellular calcium transfer pathways, as well as an overexpression of genes encoding organic matrix proteins.

Conclusion

The shell of chicken eggs is a complex structure and although it is not eaten, it is crucial to allow the marketing of eggs. It is therefore the object of particular attention from the point of view of breeding to improve its mechanical properties, as well as for scientists to understand its calcification. During the last 20 years, considerable progress has been made in terms of understanding the mechanisms of regulation, mineral supply and molecular actors of its biomineralization, which are at the origin of the mechanical properties of this natural biomaterial. This knowledge is already being used by breeders to integrate this component into the precision of genomic selection and to allow new gains other than those integrating shell mass alone. Recently, it has also been shown that shell formation is dependent on vitamin D and its form of intake. All this knowledge opens an important field of perspectives for a genetic-nutrition interaction in order to improve shell quality in a sustainable way during production cycles maintained at advanced ages and in a strong context of evolution of the production systems of eggs for consumption towards alternative breeding.

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Towards sequencing the genomes for all extant bird species, the progress of B10K project (G. Zhang)

Guojie ZHANG
(guojie.zhang@bio.ku.dk)

Villum Center for Biodiversity Genomics, Section for Ecology and Evolution, Department of Biology, University of Copenhagen, Copenhagen 2100, Denmark

The Bird 10,000 Genome Project (B10K) aims to sequence representative genomes for all extant bird species. The first milestone of the B10K Project, to sequence representative genomes for all avian orders with 48 species, was published in 2014 as a special issue in Science. In November 2020, B10K achieved another milestone and reported the family phase in Nature with a cover image, where we released new genomes for 268 bird species representing 92% of avian families. Combined with avian genomes produced by other researchers, whole-genome data for 363 avian species were reported on in this study, all of which are publicly available and free to use. Since then, the consortium and the project have been making swift progress. We are now at the genus phase and will produce reference genomes for over 2200 species representing over 90% of all genus. Our ultimate goal is to finish the sequencing for all species phase within next few years. As we work to complete the genus phase of the project, the number of new collaborators (individuals and institutions) has grown sharply. Some are providing genetic samples from biorepositories while others are actively engaged in fieldwork or assisting with computational analyses. In this presentation, I will give an introduction of the B10K organization and the latest progress of the project.

Waterfowls

A selective review of Pekin duck nutrition (L. Adeola)

Layi ADEOLA
O. Adeola (1) and X. Chen (2)
(1) Purdue University, West Lafayette, IN, USA; (2) NUTRIBINS, LLC., CA, USA 5
Corresponding author: ladeola@purdue.edu

Abbreviated Title: Nutrition of meat ducks

Summary

With the continuing growth of the global duck industry and advances in genetic selection of modern ducks, formulation consideration across feedstuff resources to accurately meet the bird's nutrient requirements continues to be a challenge. This article discusses research outcomes on duck nutrition, with a focus on Pekin ducks, the predominant commercial breed. Recent research that re-evaluated nutrient requirements in meat ducks has shown shifts in energy, crude protein, and amino acid requirements across genetics and production goals, as these nutrients not only affect growth performance, but also influence carcass composition. Vitamin and mineral concentrations to optimize skeletal health in meat ducks and improve reproductive performance in duck breeders have also been evaluated, and the importance of maternal nutrition in duckling performance is evident. Recent research has shown great potential of novel feed additives, which may offer new opportunities to optimize production efficiency by improving nutrient utilization and health status of ducks. Producers must be more mindful of the available options and effectiveness of nutritional modification. Such nutritional modification should be implemented systemically to embrace a pragmatic balance of breeding, nutrition, and management to ultimately meet their specific market needs and bring profitable advancement to the global duck industry.

Key words: Feed additive, ingredient, nutrient requirement, nutrient utilization, Pekin duck

Introduction

With the steady increasing demand for animal protein to meet world population needs, poultry meat becomes one of the top animal protein sources because of their fast growth and high efficiency. Among poultry species, ducks are more efficient compared to broilers with growing market potential. The global meat duck production increased from 932 million head in 2000 to 1.1 billion head in 2018. Asia shared 88.2% of the global output with mainland China representing approximately 80% of the regional production and continue to contribute more than 65% of the global total (FAO STAT, 2018).

In current commercial meat duck production, Pekin duck (*Anas domesticus*) is undoubtedly the predominant breed (followed by Muscovy and Mule duck), primarily due to its rapid growth rate. Tremendous improvements have been made in through genetic selection, which has been mainly driven by the demand for faster growth rate, higher breast meat yield, and improved feed conversion ratio in the past few decades. From 1980 to 2009, feed efficiency of Maple Leaf Farms (MLF) ducks, the largest primary breeder and producer of White Pekin ducks in North America, has improved from 0.32 to 0.59, live weight from 2.8 to 3.01 kg, with an increase in carcass yield from 71 to 74%. Additionally, boneless skinless breast yields almost doubled from 11.5 to 19.3% and carcass skin and fat has reduced from 33 to 20% during the same period, while market age reduced from 50 to 37 days (Zhou, 2009). With the advances in genetic selection, updating nutrient requirements for modern ducks is therefore an urgent need for nutritionists. Together with the ever-changing global market and trading situations, it is not surprising that understanding the nutritional value of different feedstuff resources existed in different geographic regions is important. Additionally, the trend of antibiotic-free production offers new opportunities for novel feed additives to optimize efficiency. Understanding how these new tools work and how to utilize them in Pekin duck production may assist in the sustainable and efficient growth of the industry. This treatise discusses a few (given the imposed limitation in length) new research outcomes in Pekin duck nutrition in regard of nutrient requirements, nutrient sources, and nutrient utilization opportunities via various feed additives.

1. Energy

1.1. Requirement for Energy

Like any other animal, ducks require an amount of energy for maintenance (basal metabolism, body temperature regulation, and regular activity) and growth. Commercial meat ducks are typically fed a 2- phase: a starter diet from hatch to 14 or 21 days (d) of age followed by a grower diet; a 3rd -phase finisher diet can be fed if the ducks are to be held for additional days after 35 or 42 d. For any diets during the rearing period, energy is the most expensive component. Requirement of meat ducks for energy are typically expressed as dietary metabolizable energy (ME), the portion of gross energy in the feed remaining after excreta energy loss. Over the past few decades, the ME recommendation for Pekin ducks generally fall within the range of 2825 to 3000 kcal/kg for starters and 3000 to 3100 kcal/kg for growers (NRC, 1994; Leeson and Summers, 2009; Adeola, 2006; Fan et al., 2008). A few reports (Xie et al., 2010; Chen et al., 2017; Zeng et al., 2015; Liu et al., 2019) showed a shift to 120 to 350 kcal/kg higher ME requirement compared to that from NRC (1994). This shift may be partially due to the continued advance in genetic selection for a faster-growing duck and dietary amino acids.

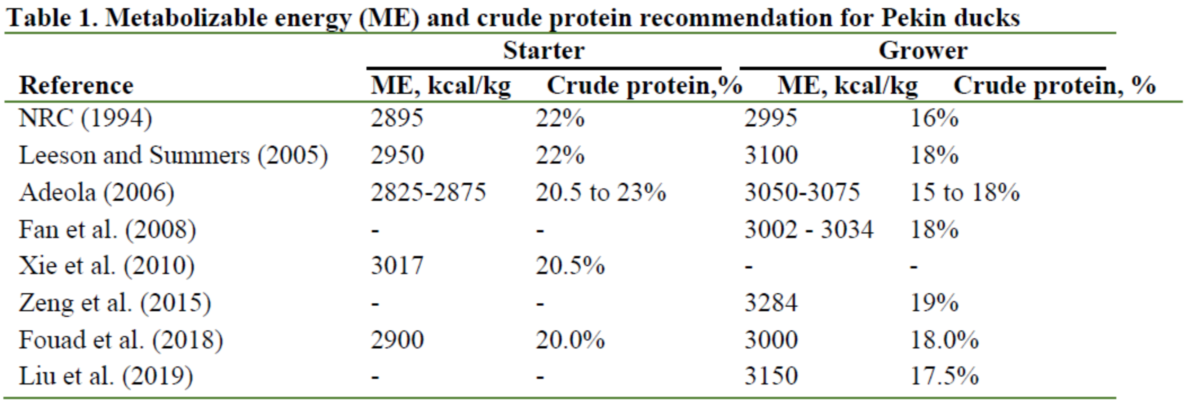


Table 1. Metabolizable energy (ME) and crude protein recommendation for Pekin ducks

	Starter		Grower	
Reference	ME, kcal/kg	Crude protein,%	ME, kcal/kg	Crude protein, %
NRC (1994)	2895	22%	2995	16%
Leeson and Summers (2005)	2950	22%	3100	18%
Adeola (2006)	2825-2875	20.5 to 23%	3050-3075	15 to 18%
Fan et al. (2008)	-	-	3002 - 3034	18%
Xie et al. (2010)	3017	20.5%	-	-
Zeng et al. (2015)	-	-	3284	19%
Fouad et al. (2018)	2900	20.0%	3000	18.0%
Liu et al. (2019)	-	-	3150	17.5%

It is well accepted that ducks are able to regulate energy intake via feed intake, and is considered non-responsive to variations in dietary energy level (Fan et al., 2008; Xie et al., 2010). Adeola (2006) reported that significant improvements in body weight gains and efficiency of feed utilization were not observed when diets are formulated to contain more than 3000 kcal/kg. Meat ducks fed low-energy diet can increase feed intake to ensure growth requirement. However, when dietary ME is high, ducks will reduce feed intake and thus have a lower intake of protein & amino acid, the building blocks for body protein deposition. Therefore, amino acid levels should be adjusted accordingly with dietary ME change; if amino acid profile is not balanced with dietary ME, protein deposition is likely to be affected. Therefore, it is key to balance dietary ME and amino acids; these observations also highlight the importance to pay attention to the protein and amino acid levels when evaluating and reviewing ME requirement in ducks.

1.2. Energy sources

Dietary energy is stored in the form of carbohydrate, protein, and lipids. Compared to broilers, there is relatively limited research in ducks on nutritional evaluation of ingredient, and therefore, duck diet formulations often use ME values obtained from broilers, yet the accuracy of using such data is questionable. Several studies have been conducted to determine the ME values of a variety of grains, meals and by-products for ducks (Adeola, 2006; Hoai et al., 2011). A general trend observed is that energy value for most of the grains are greater than those in meals resulting after oil extraction, and the hierarchy of energy values for the grains and meal is the same for broilers and ducks (Baéza, 2019). Hong et al. (2001, 2002) confirmed the assumption in Pekin ducks that ME values of individual feedstuffs are additive for barley-canola meal diet and corn-wheat red dog-SBM diet.

1.3. Energy utilization

High levels of non-starch polysaccharides (NSP) in grains are known to reduce growth performance, primarily through its ability increase digesta viscosity which leads to a subsequent decrease in energy utilization. Several recent researches have focused on the potential of NSP enzyme supplementation to improve energy utilization and performance in ducks. When ducks are fed diets containing low- or high-viscosity wheat supplemented with xylanase, high viscosity wheat decreased TME and xylanase supplementation improved energy digestibility and TME more so for high-than low-viscosity wheat (Adeola and Bedford, 2004). These observations demonstrated that high viscosity was the predominant cause for the low energy utilization from NSP, and that the beneficial effect of supplementing xylanase comes primarily from reducing viscosity. Park et al. (2019a, b) reported that β-mannanase, an NSP enzyme that breaks down the beta-mannan backbone chain, in diets of starter ducks from 1 to 21 d. improved growth performance through reducing digesta viscosity and improving nutrient utilization. Using the abovementioned precision-fed total collection method, Adeola (2007) found improvements in energy and nutrient utilization in ducks from a cocktail of xylanase, amylase, and protease. Enzyme cocktail of xylanase + β-glucanase + phytase was also reported to have the potential to compensate AME by 100 kcal/kg, along with other nutrients (amino acids, P, and Ca) in ducks from 1 to 35 d of age (Zeng et al., 2015).

Most enzyme efficiency studies in poultry were conducted with broilers; information regarding ducks is still scarce. Based on a recent review (Park and Carey, 2019), there were only 8 studies since 2000 that evaluated effects of xylanase, protease, or multi-enzyme in meat ducks, most showing improvements in growth performance through increased nutrient utilization. With the wide variety of enzymes on the market today, how to improve energy utilization through the application of specific enzyme or enzyme complex in ducks is worth further examination.

2. Protein and Amino Acids

2.1. Requirement for crude protein and amino acids

Considerable variation exists in the industry on how much crude protein is fed. The NRC (1994) recommended 22 and 16% for starter and grower ducks, respectively, whereas Zeng et al. (2015) recommended 19% in grower ducks to obtain the best growth performance and carcass traits. Crude protein level essentially depends on the varied demands for end product composition. Western nations predominantly use duck breast meat and prefer minimal fat content, whereas Asian countries prefer whole duck product with higher subcutaneous fat content to create the crispy fleshy texture. Therefore, there is not a single standard for duck carcass characteristics and thus a better understanding of specific market requirements is necessary.

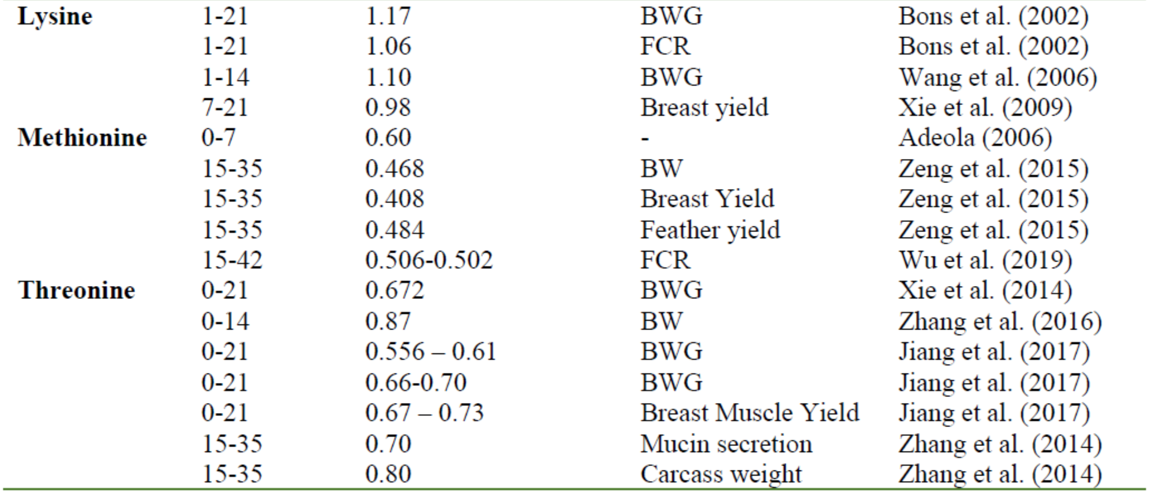
Indeed, manipulating dietary protein and amino acid profile together with ME levels can lead to very different compositions within strain. The abovementioned study by Zeng et al. (2015) serves as a nice example in which grower ducks were fed 3 dietary ME (2818, 3057, and 3296 kcal/kg) with 3 crude protein (CP) concentrations (15, 17, and 19%) in a 3 x 3 factorial arrangement. Significant interactions were found between dietary ME and CP on BW gain, feed intake, and breast meat weight on d 35, where the best BW gain and feed conversion ratio was obtained when ducks were fed 3296 kcal/kg ME and 19% CP. Carcass traits were also affected by dietary energy and amino acid profile, where the highest breast meat weight was observed in ducks fed 3057 kcal/kg ME with 19% CP. Increasing dietary CP concentration from 15 to 19% showed a significant main effect of increasing breast meat yield from 18 to 20%, with a decrease of breast skin and fat yield from 6.78 to 6.18%. Conversely, increasing ME increased dressing percentage, breast skin and fat yield (from 6.2 to 6.9%), but decreased breast meat yield (from 19.4 to 18.4%).

The purpose of adding protein to the diet is to provide amino acids. Essential amino acids are often the research focus but, it is important to note that dietary supply of non-essential amino acids must be adequate to satisfy the non-essential nitrogen need, especially in the case of low-CP diet. In corn soybean meal diet, methionine is likely to be the first limiting amino acid for ducks, followed by lysine, threonine, and tryptophan. Adeola (2006) suggested that ducks do not require more than 0.60% Met during the first week of hatch. In grower ducks, optimal Met was 0.468, 0.408, and 0.484% for BW, breast meat yield, and feather; increases in Met improved carcass and breast meat yield and led to reduced breast skin and subcutaneous fat (Zeng et al., 2015). These levels were like what was reported by Wu et al. (2019) in grower ducks. Also, Met requirement was affected by dietary ME level, and that Met/ME ratio remain constant.

Lysine is often the second limiting amino acids in a duck diet, and requirement in starter ducks from recent studies ranged from 0.98 to 1.10% (Table 2). Adeola (2006) suggested starter ducks do not require more than 1.2% lysine. Requirement for threonine, often the third limiting amino acid, and in addition to its impact on duck performance, is also critical for maintain intestinal structure and function, as evident by increased small intestine villi height with increasing dietary Thr level (Xie et al., 2014) and a higher requirement to maximize mucin secretion compared to that maximizing BWG 165 (Zhang et al., 2016). No values for Thr requirement for Pekin ducks were established in NRC (1994). Recently, Zhang et al. (2016) recommended 0.87%, 0.90, and 0.98% for optimal BWG, mucin secretion, and FCR in starter ducks (0 to 14 d); these values were much higher compared to what was recommended by Xie et al. (2014) at 0.67% or by Adeola (2006) at 0.62 to 0.76%. The discrepancy might be due to the varied crude protein, Lys, and ME level used in each study, and thus Thr requirements need to be adjusted accordingly. Indeed, when starter ducks (0 to 21 d) were fed 6 graded Thr levels with 2 dietary crude protein levels (17.7 and 20.1%), the Thr requirement was 0.610% at 172 20.9% CP and 0.556% at 17.7% CP when expressed as % of diet, and was 2.931% at 20.9% CP and 3.146% at 17.7% CP when expressed as % of dietary CP, both showed that dietary CP affected Thr requirement, whilst when expressed as % of dietary Lys, the Thr requirement was not affected by dietary CP (53.0% at 20.89% CP and 52.0% at 17.73% CP). Dietary CP can cause variations in the Thr requirement of Pekin ducks, but expressing the Thr requirement as a % of dietary Lys can reduce this variation (Jiang et al., 2015; 2017). There has been relatively limited research on Thr requirement in grower ducks. A range from 0.70% to maximize mucin secretion to 0.80% to maximize carcass weight may be required (Zhang et al., 2014) but a low of 0.62% to minimize mortality and a high of 0.73% to maximize mucin secretion.

On another note, amino acid requirements for ducks are still mostly being estimated on a total basis, but not on a standardized ileal digestible amino acid basis; the latter has been widely used in broilers and pigs as a more accurate base for formulation. Indeed, 100% of amino acids ingested is not digested and absorbed by the bird. For instance, apparent ileal digestibility of Thr was between 73 and 83% in grower Pekin ducks when diet contained 0.57 to 0.80% Thr (Zhang et al., 2014). Clearly, research to establish standardized ileal digestible amino acid requirements and for amino acids other than Met, Lys, and Thr in Pekin ducks warrants further attention.

Table 2. Dietary lysine, methionine, and threonine requirement recommendation



Amino acid	Duck age, d	Recommendation, %	Optimal response	Reference
Lysine	1-21	1.17	BWG	Bons et al. (2002)
	1-21	1.06	FCR	Bons et al. (2002)
	1-14	1.10	BWG	Wang et al. (2006)
	7-21	0.98	Breast yield	Xie et al. (2009)
Methionine	0-7	0.60	-	Adeola (2006)
	15-35	0.468	BW	Zeng et al. (2015)
	15-35	0.408	Breast Yield	Zeng et al. (2015)
	15-35	0.484	Feather yield	Zeng et al. (2015)
	15-42	0.506-0.502	FCR	Wu et al. (2019)
Threonine	0-21	0.672	BWG	Xie et al. (2014)
	0-14	0.87	BW	Zhang et al. (2016)
	0-21	0.556 – 0.61	BWG	Jiang et al. (2017)
	0-21	0.66-0.70	BWG	Jiang et al. (2017)
	0-21	0.67 – 0.73	Breast Muscle Yield	Jiang et al. (2017)
	15-35	0.70	Mucin secretion	Zhang et al. (2014)
	15-35	0.80	Carcass weight	Zhang et al. (2014)

2.2. Protein sources

Whereas soybean meal (SBM) and wheat are the predominant protein sources in North America and Europe for duck feed formulation, a variety of protein sources has traditionally been used in Asian countries. Canola meal (CM) is arguably the third most used protein ingredient for duck. Historically, the use of CM has been limited in poultry diets due primarily to the presence of glucosinolates that reduce feed intake and growth rate and increase mortality and liver damage. Although the concentrations of glucosinolates have already been reduced to low levels in CM compared to traditional rapeseed meal, a recent study showed that as dietary canola meal level reached 15% (3.46 to 200 5.31 μmol glucosinolates/g), performance of ducklings at 7 to 15 d of age were decreased, but no differences were noticed from days 15 to 21. This may indicate a greater tolerance of CM in older ducks (Zhu et al. 2019). In the same study, another source of rapeseed meal containing a higher glucosinolates was evaluated in grower ducks, yet results showed growth depression by as low as 5% inclusion rate. Therefore, it appears that the upper limit of using canola meal or rapeseed meal sources in feed formulation depends on the glucosinolates content and duck growth stage.

Canola meal also contains higher fiber compared with SBM, which is largely responsible for a lower total tract digestibility of crude protein and energy, and consequently a lower ME in poultry. When SBM and CM were compared in grower ducks using regression method, apparent ileal digestibility of crude protein and several amino acids were significantly higher for SBM than CM (83.3 vs. 77.6% for crude protein, respectively; Kong and Adeola, 2013). Interestingly, although ducks seem to utilize energy of canola meal more efficiently than chickens, comparable amino acid digestibility was found between broilers and ducks (Hong et al., 2001; Kong and Adeola, 2013). Another protein source evaluated is DDGS. Adeola (2015) evaluated N and amino acid digestibility of DDGS in grower ducks and reported an apparent ileal N digestibility of 77.35%, which was lower than SBM but was similar to corn, wheat, and CM, and higher than meat and bone meal. Growth performance and carcass yield of grower ducks are not adversely affected by up to 30% dietary DDGS and has great potential to be an alternative protein source for ducks.

The use of cottonseed meal is limited by the presence of free gossypol that induce liver toxicity in poultry and ducks are more sensitive to dietary gossypol compared to broilers. When grower ducks were fed graded 0 to 100% cottonseed meal in replacement of SBM (containing 0 to 153 mg free gossypol /kg diet), performance remained unaffected at 75 to 111 mg/kg gossypol diet; but serum globulin concentration was more sensitive to gossypol and was reduced at 36 mg/kg gossypol diet. It was concluded that grower ducks fed 36 mg free gossypol/kg (5.83% cottonseed meal of diet) diet had a normal performance and histological structure of liver, and muscle (breast and leg) had no residue of gossypol (Zeng et al., 2015).

3. Vitamins and Minerals

3.1. Requirement for vitamins and minerals

Recommendations for vitamin and minerals in Pekin ducks from NRC (1994), DSM (2016) and other review papers are in (NRC (1994), Adeola, 2006; Leeson and Summer, 2009; and Fouad et al., 2018). Intensive selection for growth rate in ducks has resulted in an unwanted increase of skeletal problems in duck production, and a better understanding of bone growth and mineralization in today's ducks is thus an essential first step to develop counteractive strategies. A recent duck study revealed that tibia displayed rapid bone growth from days 1 to 35 of age and mineralization from d 1 to 42. The fat-free weight, ash, calcium (Ca) and phosphorus (P) content of tibia increased rapidly from 1 to 42 d and reached its plateau from 42 d onward, but density and tibia-breaking strength of tibia increased in a regular manner until 42 d (Zhang et al., 2019). Meat ducks are typically processed at 32 to 35 d of age before mineralization is completed, therefore, improving mineralization during this short lifespan of meat ducks may help alleviate skeletal health issues.

The NRC (1994) recommended 0.65% Ca and 0.40% non-phytate P for starter ducks, and 0.60% Ca and 0.30% for grower ducks, which has been extrapolated from other species. Later, Rush et al. (2005) reported that maximal duckling growth was realized when 0.95% Ca was fed, and Xie et al. (2009) reported a requirement of 0.81% in starter ducks; both exceeding the NRC recommendation, but P requirement stayed relatively similar to that of NRC (Xie et al., 2009). Conversely, a later study by Xie et al. (2016) showed a higher P requirement where the nPP requirements of ducks from hatch to 12 d of age increased as dietary Ca increased and were 0.49 and 0.75% nPP at 0.75 and 1.15% Ca, respectively. In grower ducks, higher Ca requirement of 0.66 to 0.72% Ca at 0.37% P was reported (Xie et al., 2009b). It is important to note that higher concentration of Ca in the diet may decrease phytate-P hydrolysis due to Ca chelation of the phytin molecule, therefore providing an optimal Ca to P ratio is critical, as a high Ca:P ratio can lead to bone mineral loss, which could be related to elevated serum alkaline phosphatase activity and the reduced utilization of energy, Ca, and P (Zhu et al., 2018)

Vitamin D3 is an important part of bone growth and mineralization, with a main role of raising the plasma concentrations of Ca and P to normal levels so that these minerals can contribute to their vital roles in bone metabolism. The NRC (1994) recommendation of 400 IU/kg is extremely low compared to current industry normal that can be up to 6000 IU/kg or higher depending on country legislation limit. In recently years, the use of 25-hydroxychlecalciferol (25-OH-D3), an intermediate metabolite of vitamin D3, gained popularity in poultry due to its better efficiency. Limited research on 25-OH-D3 in ducks showed that supplementing 25-OH-D3 at 0.069 mg/kg significantly increased sternal mass by suppressing bone resorption (Zhang et al., 2020).

Mineral Utilization

As in broilers, the use of microbial phytase in duck diets has become an effective tool in the industry in recent years. Adding phytase has been shown to increase phytate hydrolysis and availability of phosphorus in ducks (Orban et al., 1999; Rodehutscord et al., 2006). In practical feed formulation, it is important to know the P equivalency of phytase; but surprisingly scarce information is available for ducks. Adeola (2010) showed that the addition of 500, 1,000, and 1,500 units of phytase to a low-P diet released 0.453, 0.847, and 1.242 g inorganic P/kg of diet from 7 to 17 d in ducks, respectively. Consistently, feeding the low-P diet to ducks reduced BWG and FI compared with the positive control diet in both starter and grower phases, and supplementing phytase to the low-P diet resulted improvements in growth performance and tibia ash, which may partly attribute to the increased ileal digestibility and total tract retention of P in the phytase-supplemented group (Adeola, 2018). In the same study, a supra dose of phytase at 15,000 units/kg of diet did not have deleterious effects on ducks.

4. Lipids

The Pekin duck is unique in that carcass traits are vital for the desired end-product characteristics for Pekin roasting duck. It is well accepted that Pekin ducks weighing 3.0 to 3.3 kg BW at 40 d of age with more than 37% subcutaneous fat yield were suitable (Ao and Kim, 2020). Clearly, dietary lipid level and lipid sources can modulate performance and carcass traits in Pekin ducks. When grower ducks were fed soybean oil, palm oil, or duck fat, ducks fed soybean oil diet had the best growth performance and taste scores for roasting, whereas the duck fat was better in abdominal fat and subcutaneous fat yield than soybean oil and palm oil under the same nutritional level (3250 kcal/kg ME and 16.5% CP, Ao and Kim, 2020). Conversely, Liu et al. (2019) showed that palm oil was more preferable compared with soybean oil and lard in Pekin ducks for abdominal fat and subcutaneous fat content under the same ME:crude protein (CP) ratio from 15 to 40 d of age (2950 kcal/kg ME and 16.5%CP); and the addition of different lipid sources did not affect growth performance in ducks fed soybean oil or poultry fat (2880 kcal/kg ME and CP 18.2%; Hu et al.,2019). The inconsistency may likely be attributed to different supplemental lipid level, ME and CP levels, and ME: CP ratio.

Optimum dietary lipid digestion requires bile salts to emulsify lipid globules into smaller droplets, with activated lipase initiating digestion. Furthermore, bile salts are needed to form micelles and facilitate lipid absorption into the enterocytes. Emulsifier composed of glycerin monosterarate and polyoxythylene sorbitol mono-fatty acid ester at 200 mg/kg of a low-fat diet improved growth performance, carcass traits, and lipid metabolism, and promoted digestion and absorption of fat in grower ducks (Hu et al., 2019). A lecithin emulsifier added at 0.1% in grower ducks also improved growth performance, but carcass traits were not affected. Additionally, lipase and exogenous bile acid supplementation have seen beneficial effects in broilers (Want et al., 2016; Lai et al., 2018), but no information is available in Pekin ducks to date.

5. Conclusion

Continued advances in genetic selection of Pekin ducks have brought the global meat duck industry a fast-growing and efficient bird that has great potential to help meet the need for animal proteins worldwide. Recent research in Pekin ducks has provided updated information regarding nutrient requirement for energy, crude protein, amino acids, and vitamins and minerals. In general, shifts to higher requirements compared to NRC (1994) were observed, yet discrepancies still exist that may be due to different Pekin duck strains used and experimental feed formulation. It is important to balance dietary ME, crude protein, and amino acid levels, along with careful selection of nutrient sources, as they not only affect growth performance, but also modulate carcass traits. Additionally, optimizing skeletal health require careful attention to vitamin and mineral levels. Maternal nutritional program may offer a new approach to promote duckling performance and health. For traditional and non-traditional feedstuffs used in duck diet, the presence and impact of antinutritional factors should be monitored; and future nutritional evaluation of feedstuffs should be conducted on a standardized ileal digestible amino acid basis. A variety of novel feed additives that modulate gut functions and health may provide great opportunity for sustainable, efficient growth of the industry. Indeed, nutritional modification can be effective to produce output within strain to satisfy specific markets in duck production. More understanding of nutrient requirement and application of novel nutritional tools in ducks require further research.

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Quality of egg products

Promotion of local hen breeds: the quality of eggs (C. Alamprese)

Cristina ALAMPRESE

Università degli Studi di Milano, Italy

cristina.alamprese@unimi.it

Promotion of local chicken breeds is important in a sustainable perspective to support biodiversity, local economies, and farm multi-functionality, while providing consumers with poultry products of high ethical value and quality. Moreover, local breeds are suitable for alternative rearing systems, thus contributing to the environmental impact reduction. However, a major concern is the low production efficiency of local breeds, which can be overcome by crossbreeding with high performance strains, but the quality of the obtained poultry products must be checked and validated. In this context, the aim of the present work was the quality assessment of the eggs produced by two Italian local breeds (Bionda Piemontese, BP; Robusta Maculata, RM), their crossbreeds with the commercial hybrid Sasso (BPxS and RMxS genotypes), and the commercial strain Lohmann brown (L) considered as the reference.

Welfare of layers

Recent progress on feather pecking behavior and perspectives to avoid beak trimming (B. Rodenburg)

Bas RODENBURG

T.B. Rodenburg (1)

(1) Animals in Science and Society, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 2, 3584 CM Utrecht, The Netherlands

Corresponding author: t.b.rodenburg@uu.nl

Abbreviated title: Prevention of feather pecking

Summary

Feather pecking is the pecking at- and pulling out of feathers of other birds. Feather pecking can be a problem in all current laying hen housing systems, but it is the most difficult to control in non-cage systems. Beak trimming has been a common measure to limit the damage that birds can inflict on each other. However, the practice of beak trimming is also under scrutiny, because it does not offer a real solution to the problem of feather pecking and because it can cause both acute and chronic pain. EU egg production is moving towards non-cage systems, in which increasingly birds with intact beaks are kept. To minimize the risk of feather pecking developing in these systems, attention is needed throughout the whole breeding and production chain. Regarding breeding, increasing attention has been given to prevention of feather pecking in recent years. Further, evidence has been found that stress in the parent stock can result in early development of severe feather pecking in the offspring. In rearing flocks, attention to managing fear and stress is needed, but also ensuring access to a good quality litter material has been shown to be very important. From chick to pullet to laying hen, birds go through many transitions and are moved from the rearing farm to the laying farm. Ensuring that these transitions are smooth helps to prevent outbreaks of feather pecking. In conclusion, to minimize the risk of feather pecking, resilient birds are needed, that lack a strong propensity to peck at other birds. They should be kept in a stimulating environment, that provides opportunities for foraging and exploratory behaviour. These approaches should help to prevent outbreaks of feather pecking in flocks with intact beaks.

Key words: Feather pecking, beak trimming, laying hen, breeding, rearing, management

Introduction

Feather pecking is the pecking at- and pulling out of feathers of other birds. Feather pecking can be a problem in all current laying hen housing systems, but it is the most difficult to control in non-cage systems (Rodenburg et al., 2013). Beak trimming has been a common measure to limit the damage that birds can inflict on each other. However, the practice of beak trimming is also under scrutiny, because it does not offer a real solution to the problem of feather pecking and because it can cause both acute and chronic pain (Marchant-Forde et al., 2008). EU egg production is moving towards non-cage systems, in which increasingly birds with intact beaks are kept. To minimize the risk of feather pecking developing in these systems, attention is needed throughout the whole breeding and production chain. Experience with management of hens in non-cage systems is increasing, leading to improved performance and reduced mortality compared with the previous two decades. This is illustrated by a recent meta-analysis by Schuck-Paim et al (2021) of over 6,000 flocks worldwide. They show that mortality levels in non-cage systems can be brought to a similar level to that in cage systems, once the producers have a sufficient level of experience to work with the system successfully. It is also clear that non-cage systems are more challenging to manage than cage systems and that special attention is needed to prevent feather pecking and cannibalism outbreaks or to reduce their impact. The aim of this paper is to provide an overview on recent progress on prevention of feather pecking and perspectives to avoid beak trimming.

Breeding

Breeding plays an important role in the prevention of feather pecking. It is known that genetic variation exists in the propensity to develop feather pecking (Rodenburg et al., 2013). For instance, birds with a stronger pecking motivation or hyperactive birds seem more at risk to develop feather pecking (Kjaer, 2009). Breeders have developed methods to screen their selection candidates for their propensity to show feather pecking behaviour. This is for instance done by housing the sisters of the selection candidates in family groups in cage systems. Feather damage and survival of the sisters is then included in the selection decision and only selection candidates are selected with low or no mortality and a good plumage condition in the sister group (Ellen et al., 2014). New breeding methods, that include indirect genetic effects, allow to model genetic effects of group members on traits like plumage condition, allowing breeders to separate genotypes of peckers and victims (Biscarini et al., 2010). One challenge that remains in breeding, is the fact that the majority of the breeding stock is still kept in small groups in cage systems, while the commercial hybrids are kept in large flocks in non-cage systems. Tools are needed to monitor individual performance of hens in these large flocks, to allow collection of relevant phenotypes under commercial conditions. Here, sensor technology and computer vision will play an important role, as our possibilities to monitor behaviour of individual animals are rapidly increasing (Ellen et al., 2019).

Parent stock

Laying hen parent stock in Europe is usually housed in single-tier systems and hens are kept with approximately 10% roosters. Laying hen parent stock have received little attention in research, and this may need to change. In a Dutch project focusing on reducing feather pecking, we found that feather damage and basal corticosterone levels in the parent stock were predictive of the performance of the offspring: offspring from parents with severe feather damage at 40 weeks of age and increased corticosterone levels were more likely to show severe feather pecking already in the first weeks of the rearing period (de Haas et al., 2014b). In that sense, it may be worthwhile to invest in management of the parent stock, as we know that once a flock has developed feather pecking, it is very difficult to stop this behaviour later in life. It is also clear that there are major differences between birds from a white genetic origin and birds from a brown genetic origin: the relationship between stress in the parents and feather pecking in the offspring was only observed in the white flocks included in the study, not in the brown. This fits with the observation that white flocks are in general more fearful and more stress sensitive than brown flocks, and that this may negatively affect performance of white flocks (de Haas et al., 2014a).

Rearing flocks

The rearing period, the first 17 weeks of a laying hen's life, has strong effects on performance later in life (Janczak and Riber, 2015; De Haas et al., 2021). In the Dutch feather pecking project, we found that access to litter or to other pecking enrichments was critical in prevention of feather pecking. In rearing aviaries, pullets are generally locked into the aviary cages for the first five weeks of life. They are reared in chick paper, which allows feed, feces, down feathers and dust to aggregate on the paper, providing litter material to the birds. In our study, approximately half of the rearing farmers removed this paper approximately two weeks before releasing the hens, while the other half did not. Flocks that had continuous access to litter material on the paper were much less likely to develop feather pecking during the rearing period than flocks that experienced a period of litter removal. This effect was even stronger if extra litter was supplied on the floor when the pullets were released in the aviary system (de Haas et al., 2014b). Again, a major difference between brown and white genetic origins was observed, as this strong effect of litter access was only seen in the brown rearing flocks and not in the white. We concluded that pullets should be reared in a stimulating environment with suitable litter for foraging and pecking and should be managed in such a way that they become resilient to human activities and other events. They should be monitored closely and should be offered environmental enrichment (roughage, pecking blocks, straw bales) when first signs of feather pecking are detected. Also, nutritional advice can be sought to rule out shortages in the diet that may increase the risk of feather pecking.

Laying flocks

As the final link in the chain, of course the laying hen farmer also plays pivotal role in the prevention of feather pecking. In the Dutch feather pecking project, we investigated which factors from the rearing period and from the laying period were predictive of feather damage at 40 weeks of age. We found that high levels of severe feather pecking at five weeks of age and elevated fear of humans during rearing were predictive of feather damage during lay. Risk factors during lay were large flock size, floor housing instead of aviary housing and standard management instead of adjusted management. Adjusted management included a radio playing in the house (to reduce fear), supply of pecking blocks and supply of roughage (such as alfalfa hay) (de Haas et al., 2014a). These results underline that bird management during the laying period is very important to prevent problems with feather pecking. Currently, the European egg market is transitioning towards eggs from non-cage systems. Some countries already have the majority of hens in non-cage systems, while other countries still mainly have hens in furnished cages. In the EU pilot project Best Practice Hens (www.bestpracticehens.eu), we aim to bring together knowledge on how to successfully keep hens in non-cage systems, for companies and farmers that are interested to make the transition. The project will bring together best practices for both rearing farms and laying farms and will include aspects aimed at indoor, free range and organic production. Especially for topics such as prevention of feather pecking, these types of knowledge exchange between countries are pivotal. This also became clear in a recent workshop on stimulating the transition to non-cage systems (Rodenburg et al., 2022).

Perspectives to avoid beak trimming

Consumers and NGOs are increasingly also asking for future egg production systems in which laying hens can be kept with intact beaks, avoiding the practice of beak trimming. In some European countries, such as Germany and The Netherlands, beak trimming was recently banned. During the transition phase, a lot of attention was given to prevention of feather pecking and cannibalism in flocks with intact beaks. This has paid off, in the sense that the transition to intact beaks has been quite smooth and major problems with pecking damage and mortality have not been reported. Innovations may also help to further prevent problems with feather pecking in flocks with intact beaks. In the European project PPILOW (www.ppilow.eu), we study the effects of innovations during incubation and early life on feather pecking during the laying period. In this project we investigate two factors: the effects of light during incubation and the effects of provision of insect larvae during rearing. Light during incubation may result in birds that less stress sensitive and less prone to develop feather pecking. Provision of insect larvae is expected to stimulate birds to be more focused on foraging and exploring the environment and less on other hens. In the project we will also engage with farmers to investigate and test on-farm improvements that they are interested in. Previous projects that took a similar approach showed that this can be a very successful way of exchanging knowledge on prevention of feather pecking. Nicol et al. (2013) demonstrated that producers that implemented a large number of improvements on their farms to prevent feather pecking were much more successful in achieving a good performance compared with control flocks.

Conclusion

Guidance on prevention of feather pecking becomes increasingly important in an environment where hens are increasingly kept in large flocks in non-cage systems. Breeding, parent stock farms, rearing farms and laying hen farms all play an important role in the prevention of feather pecking. An effort should be made to ensure a smooth transfer of information between the different parts of the production chain, as well as between scientists and industry. A lot of scientific knowledge on feather pecking and its prevention is available, but this does not always reach the laying hen industry. Results should also be confirmed under commercial conditions to demonstrate practical relevance. In conclusion, to minimize the risk of feather pecking, resilient birds are needed, that lack a strong propensity to peck at other birds. They should be kept in a stimulating environment, that provides opportunities for foraging and exploratory behaviour. These approaches should help to prevent outbreaks of feather pecking in flocks with intact beaks.

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Mineral nutrition

Towards digestible calcium requirements and a digestible calcium system (R. Angel)

Roselina ANGEL

Name: Roselina Angel[1], Wenting Li[1], Peter Plumstead[2] 1 8127 Regents Drive,4131 Animal Sciences Building, College Park, MD, USA, 20742. Chemuniqué Pty Ltd., Lanseria, South Africa.

E mail corresponding author: rangel@umd.edu

Abbreviated tittle: Towards digestible calcium requirements

Summary

Most nutrient requirements today are based on digestible values with an available (a) or digestible (d) phosphorus (P) system is used worldwide and widespread adoption of an amino acids digestibility system. Not so for calcium (Ca). Since Ca digestibility from ingredients has been shown to be highly variable, formulation to a total (t) Ca requirement result in varying supply of digestible (d) Ca, depending on ingredients used. To account for this variation, tCa in diets have been increased to provide a safety margin when Ca digestibility from ingredients, especially limestone, is poor resulting on over formulation of Ca in diets. The application of a ratio of tCa: aP or dP results in increased use of P to maintain the ratio and, since the digestibility of Ca is not known results in a source of error. Ca is under certain pH conditions a very reactive cation that interacts directly or through chelations with phytate and this has profound impacts on the digestibility of other mineral cations, amino acids, as well the efficacy of phytase. For the animal, what is important are the amounts of Ca and P that can be digested, absorbed and potentially used growth and maintenance. To implement a system that allows us to feed as close to P requirements as possible, we need to understand dCa in ingredients and have dCa and dP recommendations for formulation. Given the drive to lower P in diets due to its non-renewable nature and always increasing cost, we must understand the digestibility of Ca in ingredients and the digestible needs of the broiler. Work in developing a digestible Ca system has been ongoing in poultry with varying success and conflicting results. This paper seeks to give a brief overview of where we are in the implementation of a digestible Ca system.

Key words: Broilers, digestible calcium and phosphorus, requirements, ingredient matrixes

Introduction

Using digestible or available nutrients values for ingredients as well as to formulate final diets has been used in poultry formulations since the 1950's. The transition to availability started with phosphorus (P) when diet formulation using total P was replaced with a calculation of available P. In the 1954 NRC for poultry, the qualification was made giving importance to the availability of P by specifying that of the 6.0 g/kg total P requirement, 5.6 g/kg needed to be from an inorganic source and assigning a 30% availability to P in plant-based ingredients. This transition was driven by the low availability of P in phytate P. Even though available P is still used extensively nowadays in commercial feed formulation, the need to for a more precise P digestibility value has led to the move to determine dP values in vivo. In the last 20 years the determination of digestible amino acid values for feed ingredients has resulted in its implementation in most formulation systems worldwide. Yet we continue to formulate a key mineral, calcium (Ca), on a total basis. To add insult to injury, we insist on trying to maintain a ratio of total (t) Ca to a form of available or digestible (d) P of around 1.8 to 2.2. Because of the highly variable Ca digestibility of the main Ca source in broiler diets, limestone (Angel, 2013, 2018, 2019a, 2019b; Anwar et al., 2015; 2016a, b & c; Anwar, 2017; Kim et al., 2019) describing the birds' requirements for these minerals as a ratio of tCa to available or dP makes no sense especially as we try to minimize the use of P in poultry formulation. Maintaining a ratio of tCa to available P or dP costs money not only in formulation but also in performance impairments, and can often lead to excess levels and poorer utilization of these minerals by the bird, increasing excretion and reducing the sustainability of our Industry.

Changing an existing and deeply rooted paradigm, such as that of the use of tCa, requires that not only Ca digestibility data of ingredients be researched, and robust data sets developed, but also the validation of these values for additivity in diverse diets that are used around the world and the subsequent implementation of these dCa values in animal performance studies under commercial conditions. Validation of additivity requires that diverse ingredient diets for different phases be formulated based on an ingredient dCa value system that in turn, will generate an expected dietary supply of dCa, with these diets fed to broilers in digestibility studies to determine if the dCa formulated is close to the dCa determined *in vivo*. For the last 12 years, work has been ongoing in developing a digestibility assay for dCa in broilers, with the subsequent determination of dCa in ingredients for broilers, as well as in developing prediction equations that allow for a good estimate of limestone dCa (Angel, 2019b; Kim et al., 2019). This work continues with recent application of some system to diet formulation and application in growth and digestibility studies (Walk et al., 2021a & b; 2022). Even though major steps have been taken towards establishing a working dCa system, large gaps still exist in developing a robust ingredient dCa data set that can be applied to the varied diet formulations for both research testing and commercial application. For a new system to be adopted it must show that it is usable and applicable and that there are benefits to its application.

Today there are some conflicting values, methods, and applications. This paper seeks to give an overview of what has been done as well as put forth some digestible Ca requirements for broilers.

Calcium digestibility methods

Interest in the need for a digestible Ca system for poultry, resurfaced in 2012. Physiological constrains due to the strong homeostasis of Ca in the body (Hurwitz, 1989; Murayama et al., 1998; Pike at al., 2007; Veum, 2010; Proszkowiec-Weglarz and Angel, 2013a) are well known and these must be considered while developing a methodology for ingredient digestible Ca determination in any vertebrate animal. In poultry, Ca digestibility methods developed so far are those of Anwar et al2015, 2016a & b and those of Proszkowiec-Weglarz et al., 2013 a, b & c and Angel et al., 2016.

Background on method determination. Preliminary work at the University of Maryland has shown that in diets with imbalances in Ca and P, digestibility of Ca as measured in the distal ileum changes after 48 hours post feeding of the imbalance diets (Proszkowiec-Weglarz et al., 2013b & c). The latter finding is fundamental to the methodology used to determine Ca digestibility of individual ingredients since using the direct method, where both Ca and P digestibility is being measured in ingredients, imbalances of Ca and P will be unavoidable. Of extreme importance, because of phytate effects on Ca utilization, the determination of Ca digestibility determined in diets devoid of phytate, generates values that are not necessarily applicable to most commercial diets that contain phytate. The need to determine digestibility in less than 48h after the start of feeding the experimental diet with the test ingredient necessitated challenging the dogma that the determination of digestibility coefficients in poultry should be done after feeding a test ingredient for 96h (Heller et al., 1930; Adedokum et al., 2007). In the most used procedure to determine ileal digestibility values, the broiler is allowed to adapt to the test diet, and then digestibility is determined of a test diet that all together is typically offered for 5 days. For the purpose of Ca digestibility determinations, any adaptation to a diet that may not contain the dCa to dP ratio needed by the chicken at that specific time, is undesirable if the information is to be used to formulate commercial diets where dCa and dP are both balanced and at or above requirements. Thus, the method must ensure that the animal is in a state where no gross imbalances between Ca and P exist and where needs are being met, at the start of the experimental period. This balance state, as related to digestive capacity, remains for up to 48 hours even under extreme imbalance between Ca and P in the diet being tested (Proszkowiec-Weglarz et al., 2013a). Proszkowiec Weglarz abd coworkers in 2013c showed that when a diet containing a chromic oxide marker, was fed up the start of feeding the test diets (0 time), chromic oxide was no longer measurable in the distal ileum 10 hours into the experimental period. The test diet containing titanium dioxide marker fed starting at 0 time reached a stable concentration after 12 hours. Thus, it is possible to feed an imbalanced Ca and P diet, for the purpose of determining dCa and dP in ingredients when determined in complex diets, for 24 to 40 h without seeing significant changes in Ca and P digestibility caused by the imbalances.

Comparisons between methods Publications from Massey University on ingredient digestible Ca values, give details of the digestibility assay conducted (Anwar et al., 2015; Anwar et al., 2016a, b & c). Calcium digestibility work is also being done on ingredients at the University of Maryland (Angel, 2013; Angel et al., 2013; Proszkowiec-Weglarz et al., 2013a, b & c; Angel et al., 2014). These two methods have similarities but also differ in fundamental aspects. The important differences between methods are the length of the study and the basal diet used. In the Massey University method (Anwar et al., 2015) animals were fed the test diets for 72 hours before digesta were collected, which, based on the work of Proszkowiec-Weglarz (2013b & c), would allow the animals to upregulate or downregulate Ca absorption if dCa: dP balance is not correct or if Ca and or P are formulated below or above requirements. The animals in the Massey university studies (Anwar et al., 2015, 2016a, b & c) were fed a diet in excess of Ca and P requirements up to the start of the test period regardless of the fact that those levels are not specified for the breed. In the University of Maryland method (Proszkowiec-Weglarz et al., 2013c) animals were fed the test diets for 32 to 38h, ensuring that the digestive capacity established by feeding a diet close to actual requirements prior to the start of the digestibility trial diet is maintained. This minimizes changes in Ca or P digestibility associated with test diet imbalances. Thus, diets with wide Ca to P ratios or inverse ratios, can be studied, without digestive capacity changes occurring due to homeostatic mechanisms.

The use of a purified diet containing no phytate (Anwar et al., 2015; 2016 a, b & c) as the basal diet will further result in very different digestibility of Ca from sources that have variable but moderate to high solubility such as limestones. Anwar (2016c) reported true ileal Ca digestibility of 71 and 43% (main effect means) for a 1 to 2 mm and a <0.5 mm particle size limestones, respectively. In recent work at the University of Maryland (Angel et al., 2019a & b) when the Ca digestibility of Ca from the same limestone with two particle sizes was determined either in a purified basal or in a basal diet containing corn (2.3 g/kg phytate P) and done in the same trial, the effect of the type of basal was significant and large. The same limestone at 0.80 and 0.15 mm geometric mean diameter (GMD) particle sizes had Ca digestibility of 84.3 and 72.9%, respectively when tested using a purified (no N, P or Ca) basal diet (Angel, 2019a). When the same limestone samples were tested using a corn basal, containing 2.3 g/kg phytate P and no phytase, the digestibility for 0.80- and 0.15-mm GMD particle size limestones were 55.4 and 33.6% respectively. These data highlight the profound impact that the presence of phytate in the basal diet has on the digestibility of Ca from limestone and, that this is related in large part to the solubility of the limestone (Kim et al., 2019). With this insight, further work is now ongoing in quantifying the impact of phytate concentration and source on limestone digestibility. A study reported by David (2019) also showed that the choice of basal had a large impact on dCa of an ingredient. It is important to note, that like Anwar (2016c), work at the University of Maryland showed that when the same limestone was tested at two particle sizes, because of the more rapid rate of solubilization with finer particle size, the digestibility of Ca in the limestone was lower in the smaller sized limestone (Angel et al 2019a).

Endogenous Ca losses reported by Anwar (2015, 2016b & c) vary depending on method used. These authors used a graded addition of 3 test meat and bone meals and determined endogenous losses from the slope of the regression. They reported

292, 123, 174 g/kg dry matter intake (DMI) losses in the same experiment to be variable with MBM source. In other studies, using the direct method, endogenous Ca losses were more consistent (Anwar et al., 2016b) and 127 mg/kg dry matter intake (Anwar et al., 2016c). In addition, Anwar (2016a) stated that data from seven studies yielded an endogenous Ca loss of 111 (14 SE) mg/kg DMI. Similarly, at the University of Maryland, over a series of eight studies where a N, P and Ca free purified diet has been used to determine non-diet related endogenous losses, the mean obtained for the eight studies was 106.8 (1.01 SE) mg/kg DMI (Proszkowiec Weglarz et al.,2013c).

Calcium digestibility in ingredients

Several studies have been published where digestibility of Ca in meat and bone meals, corn and soybean meal as well as in limestone and oyster shell and phosphates have been determined (Angel et al., 2016; Anwar, 2017; Angel, 2018, 2019a & b), with different digestibility coefficients of Ca determined between studies. Data tend to differ between determined dCa for the same ingredient as determined in the two different labs (Anwar et al., 2015; 2016 a, b & c; Angel et al., 2019), but both methods and actual ingredient nutrient contents were different. For example, Anwar reported a 45% digestibility of Ca in soybean meal while Angel (2019b) reported 56% based on 3 studies with three different soybean meal batches. Ingredient work is ongoing both to validate existing results and put forth a more robust set of values and expand ingredients to include those used in Europe and Asia.

Limestone Ca digestibility

In recent years, several research groups have shown that Ca digestibility in broilers can vary dramatically depending on the Ca source provided, the solubility and source (geology) of limestone, as well as by the diet source and level of phytate and use level of phytase in the diet (Anwar et al., 2016c; Angel, 2019; Kim et al., 2019; Taylor et al., 2019). With the knowledge that the digestibility of tCa can be significantly altered by the aforementioned dietary factors, and that the form of Ca provided and absolute amount can alter P digestibility, the specification of Ca requirements for broilers as tCa in the diet becomes obsolete, as does the adherence to a fixed ratio of tCa to available or dP. In current commercial diets where phytase is often used at doses above normal, limestone becomes the primary contributor of Ca in the diet. Limestone often contributes 50% or more of the Ca in commercial broiler diets. Thus, the importance of understanding limestone Ca digestibility, and limestone Ca interactions with phytate becomes essential if a dCa system is to work. Given the previous observations by multiple research groups that limestone particle size and solubility can affect the utilization of calcium by broilers and laying hens, as well as phosphorus digestibility and phytase efficacy in broilers, it was of interest to characterise the observed variation in particle size and solubility of limestones used in commercial feed production in Europe to quantify differences in limestone quality used in commercial poultry diets.

A study was done to characterize limestone samples from European countries, used in broiler diets (Wilkes et al., 2021). Samples (192) were received from commercial poultry companies. Samples were analysed for macro and micromineral analysis as well as particle size determination and solubility based on the method of Kim (2019). Of the 192 limestones used in broiler diets, Ca levels were generally high with an average of 37.82% with only 28 samples having <36% Ca. What is particularly striking is the lack of standardization of the particle size defined by the authors a GMDof these limestones used in broiler feed. While the average GMD was 248 µm, the standard deviation of 223 µm was almost as high as the average with a CV of almost 90%. Over 48% of these samples had a GMD below 150 µm, and 30% below 100um, reflecting the very fine nature of limestone frequently used. While there was a significant correlation (r^2 200 = 0.659) between the GMD and solubility of the limestone at 5 minutes, there are many exceptions to this generalization. For example, two limestone samples originating in the Ukraine and Poland, with similar respective particle sizes of 299 and 285 µm GMD, had dramatically different initial solubility at 5 minutes of 87% and 45%, respectively. In a similar manner, two limestone samples from different quarries in Germany had very different average GMD particle sizes of 46 µm and 250 µm but both reached 94% solubility at 5 minutes. For example, in the paper by Anwar (2016a), fine (<0.5 mm) and coarse (1-2 mm) limestone had *in-vitro* solubility of 0.60% and 0.33% and true Ca digestibility coefficients of 0.43 and 0.71, respectively. These findings demonstrated for the first time in broilers that a positive correlation existed between limestone particle size and *in-vivo* Ca digestibility, with calcium digestibility being negatively correlated to limestone solubility. Kim (2018) also showed fine limestone (0.075 mm) to have a higher *in-vitro* solubility that was supported *in-vivo* by a higher gizzard pH in 28-d old broilers fed diets with 0.8% or 1% calcium. However, in that study, the adverse effects of the fine, more rapidly soluble limestone on calcium digestibility were only observed when diets had a lower calcium level (0.6%) and no phytase. Further publications by the same group, Angel (2019), and Kim (2019) have subsequently provided greater insight into the impact that differences in limestone solubility arising from different sources (geology of limestone), or different particle size, can have on calcium digestibility in broilers. When comparing limestone from the same source, a reduction in particle size from 0.8 mm GMD to 0.15 mm GMD reduced standardized ileal digestibility (SID) of calcium from 49.2% to 38.1% (Angel, 2019a). Importantly, in the paper by Kim (2019), differences in GMD particle size alone could only explain <40% of the observed differences in calcium digestibility from limestone; and differences in limestone geology and physical/chemical characteristics were equally important to particle size in their potential effects on calcium digestibility. This observation is supported by our findings in the European limestone survey above, that limestone particle size alone was not able to adequately explain the large variation observed in *in-vitro* solubility between different samples of limestone. Initial models by Kim (2019) further showed that differences in calcium digestibility between limestones could be explained by the extent of solubility achieved at 15 and 30-minutes *in-vitro*, highlighting that limestone must be solubilised in the proventriculus/gizzard in order to be digested. In addition to differences in limestone characteristics, the group at the University of Maryland has also shown the phytate source to alter digestibility of calcium from limestone, with phytate from corn tending to be more reactive with calcium from limestone than when diets contained a mixture of corn and soybean meal (Angel, 2019a).

The implications of this work on limestone solubility go beyond dCa determinations for limestone. Solubility kinetics of limestone could potentially alter phosphorus digestibility to a far greater extent than the observed differences in Ca digestibility (Kim et al., 2018; Angel 2019; Kim et al., 2019; Taylor et al., 2019). Taylor (2019) evaluated three different sources of limestone that had been standardized to a 0.8mm GMD particle size, or simply included them in the same basal diet at the commercial particle size supplied by each limestone company. For each limestone, increasing the particle size and thereby slowing down the rate of solubilisation increased P digestibility. Of equal importance was that phytase efficacy and in particular, matrix values from phytase, were significantly affected by the source of limestone used in the diet and this is supported by the previous findings of Kim (2018). Work continues on trying to develop an equation that would allow feed mills to analyse limestone batches for solubility and particle size and provide this information to the nutritionist so that a dCa value for the specific limestone can be used in formulation. The initial work shows promise. These studies suggested that maximum phytate degradation can be achieved if phytase dose can be used accordingly based on Ca source, properties, and dietary phytate concentrations (Kim et al., 2018). Kim (2019) showed that with the use of solubility measure at 15 and 30 min and a particle size (GMD) predictions of Ca digestibility could have an r^2 of 0.98. But it is important to note that this was a preliminary study involving only 4 limestone and that true validation of the equation with sample data not used to develop the equation was not possible at the time. The authors (Kim et al., 2019) state “the prediction equations should be interpreted with care especially in cases beyond the scope of current study. Further studies involving a greater number of limestone samples are warranted to establish more accurate prediction equations. Validation of the prediction will also be necessary, using samples outside of those used to develop the prediction equations.” Additional work has been carried out that now involved more than 30 limestone samples. The new data set is showing that phytate content in the diet the limestone will be used in may be an important factor to include in the prediction equations (Angel et al., unpublished).

Calcium and phosphorus recommendations

Recent work published by David (2021) and Walk (2021 &2022) start filling in some of the blanks that existed in prior work. David (2021) used dCa ingredient values obtained in the same laboratory and with the same ingredients to formulate experimental diets and determined dCa and dP requirements from 1 to 10 days of age. They reported the requirement to be 3.32 and 4.36 to 4.78 g/kg for gain and bone mineralization, respectively with the caveat that these were determined with a fixed dP of 5 g/kg. As important as defining a requirement for dCa, this group of researchers showed additivity of dCa ingredient values. Walk (2021) determined a requirement for dCa between hatch and 10 days of 4.9 to 5.3 g/kg. Jiménez Moreno (2013a & d) reported a dCa requirement in broilers between placement and 200 g of body weight of 5.7 g/kg when dP was 5.4 g/kg.

In the starter phase, 11 to 24 d of age, Walk (2022) established that the requirement for dP to maximize tibia ash was 4.6 g/kg. Similarly, Jiménez-Moreno (2013b) reported a requirement for bone ash of 4.7 and 3.9 g/kg respectively for dCa and dP in broilers between 200 and 650 g of body weight. Between 650 and 1280 g of body weight a requirement of 4.4 and 3.1 g/kg for dCa and dP (Jiménez-Moreno et al., 2014), from 1200 to 2300 g of body weight a requirement of 3.8 and 2.5 g/kg for dCa and dP (Angel, 2019) and above 2300 g of body weight a requirement of 2.9 and 1.9 g for dCa and dP, respectively (Angel, 2019). Walk (2022) demonstrated that better performance and results were obtained when formulating to dCa vs a reference diet formulated for tCa. This type of information needs to be expanded and confirmed.

Conclusions

Significant advances have been made towards the development of a dCa system that can be implemented in feed formulation systems for poultry and commercial application. However, more information is still needed prior to the commercial implementation thereof. For future implementation in practical formulations, not only is more ingredient dCa information required to encompass more of the ingredients used worldwide, but also to increase the robustness of the system where more than one sample of each ingredient is represented in the dCa number. Further, more data on complex interactions of calcium source, phytate level and phytate source, and phytase enzyme inclusion are required. Parallel application of a dCa system with the current tCa system in commercial formulation would allow one to see differences over time of the over or under supply of dCa when we formulate for tCa. This would allow nutritionists at commercial companies to assess the implications, and potential practical benefits of formulating with tCa and start the reverse approach of formulating to dCa while generating tCa values. Specific to Ca, the goal is to supply sufficient quantities of this mineral at tissue level that are required for growth and physiological processes. Since large differences exist in the digestibility of Ca from different ingredient sources and qualities, this objective can be achieved using a dCa system but would not be possible in a tCa system. Avoiding excess of Ca, making better choices in terms of the main Ca supplying ingredient and considering the impacts of limestone solubility, will further result in better availability of P and effectiveness of phytase. Implementation of a dCa system can result in a decreased need for use of inorganic P sources in broiler diets that will reduce the diet costs and use of a nonrenewable resource as well as improve overall nutrient digestibility and performance of the birds we feed, and support the future sustainability of our Industry.

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General physiology

Neural and endocrine mechanisms that underpin feeding behaviour and metabolism (I. Dunn)

Ian DUNN

Royal (Dick) School of Veterinary Studies, Roslin Institute, University of Edinburgh, Easter Bush, UK.

Corresponding author: ian.dunn@roslin.ed.ac.uk

Abbreviated title: Control of feeding

Summary: The control of feeding and metabolism is critical to growth in poultry. Commercially there is a need to promote consumption in meat type birds and to control feed intake in breeding birds. The key to the control of feed intake and metabolic control is in the feeding centre of the hypothalamus which contains agouti-related protein (*AGRP*)/neuropeptide Y (*NPY*) and pro-opiomelanocortin (*POMC*)/cocaine and amphetamine regulated transcript (*CART*) are respectively orexigenic and anorectic. Changes in activity of the AGRP/NPY and POMC/CART neurones and their downstream effects result in changes in feed intake as well as changes in behaviour. The expression of AGRP may also be responsible for part of the genetic effects on body weight observed in lines of chickens or between the sexes. Feedback from the periphery including the gastro-intestinal tract and organs such as the pancreas are important for the ultimate drive from the AGRP/NPY and POMC/CART neurones. A number of factors, including classical molecules such as PYY, CCK, insulin, corticosterone and thyroid hormones almost certainly operate alongside adipokines and food related molecules. However, the role of endocrine signals from the periphery to control metabolism and feed intake in poultry has gaps and contradictions and borrows heavily on mammalian literature. Filling those physiological gaps is a worthy aim for future research.

Key words: hypothalamus, AGRP, POMC, feedback

The control of feeding and metabolism is critical to growth in poultry and manipulation of the system may offer future management opportunities. On one hand there is a desire to increase feed intake in birds raised for meat and favour anabolic versus catabolic processes (Tickle et al., 2018) as these are all related to growth. This may include the need to promote the consumption of feed which is nutritive but not palatable to chickens. At the opposite end of the spectrum there is a desire to control feed intake in breeding birds from heavy fast-growing meat strains to ensure the production of viable eggs and to ensure the welfare of the hens. This has been referred to as the broiler breeder paradox (Decuypere et al., 2010).

The key region in the brain for the control of feeding behaviour and metabolism is the arcuate nucleus of the hypothalamus (Boswell and Dunn, 2017). It contains two sets of neurones expressing *AGRP*)/*NPY* and *POMC*/ *CART*. These are respectively orexigenic and anorectic and integrate signals of energy status including through AMP-activated protein kinase (AMPK) (Liu et al., 2014) and controls behaviour to maintain energy balance, for example by increasing food-seeking behaviour or reducing activity. Principally from mammalian research we know that AGRP acts to antagonise the action of melanocortin, one of the possible splice products of the *POMC* gene , at the MC4 receptor to produce an appropriate physiological output. Although we lag behind mammalian research, in poultry the system appears to function in essentially the same way although there are important differences in the peripheral feedback to these neurones (Boswell and Dunn, 2015). In poultry, the expression of the orexigenic genes *NPY* and *AGRP* clearly increases with treatments that move the animal from its natural body weight to a lower weight (Dunn et al., 2013b, Dunn et al., 2015, Estienne et al., 2021, Dixon et al., 2022, Lees et al., 2017). This occurs with both natural changes in food intake and body weight, such as incubation behaviour, or imposed feed restriction as well as the administration of factors that reduce food intake and body weight. This suggests that in situations where body weight decreases as the result of 'voluntary' reductions there may be factors downstream of the AGRP/NPY neurones that inhibit food intake in the presence of the increased orexigenic drive that we assume comes from the increased expression and activity of the orexigenic neurones. However, as we will see this may simply be a matter of statistical power.

Changes in the expression of the anorectic *POMC* gene in response to changes in food availability in poultry have been harder to demonstrate. However, when large numbers of animals have been used it was possible to observe an inverse correlation between the expression of *AGRP* and *POMC* over 24 hours (Dixon et al., 2022). In situations when chickens had a 'voluntary' reduction in feed intake then an increase in both orexigenic *AGRP* and anorectic *POMC* expression was observed (Dunn et al., 2015). This may indicate that in part, the activity of the anorectic neurones are responsible for the reduced feed intake and body weight loss seen despite increased orexigenic *AGRP* expression. It was also observed that *POMC* expression was lower in red jungle fowl which have a relatively low body weight and it is tempting to speculate that this may be related to genotype (Lees et al., 2017). Further confirmation of AGRP neurones in the control of body weight in chickens comes from the correlation between *AGRP* expression and genetic variation in body weight at the locus on chicken chromosome 4 which features differences in expression of *CCKAR* and the sex chromosome. In both *AGRP* expression is higher in the faster growing genotype (Caughey et al., 2018, Dunn et al., 2013a). This led us to speculate that the activity of the AGRP/NPY neurones might form part of the genetic mechanism to determine ultimate body weight and growth rate. In fact, it is possible to observe that *AGRP* expression in chickens gives an accurate readout of how far an animal is from the body weight it would wish to achieve if it fed *ad-libitum* and not simply satiety (Dunn et al., 2013b). What is less clear in chickens is the endocrine and neural pathways that convey information to the arcuate nucleus about nutrient intake and energy balance.

This is due to the limited amount of research, the differences from the mammalian system, especially the apparent lack of a role of leptin, along with the different overlapping systems controlling feeding and metabolism peripherally (Boswell and Dunn, 2015). Understanding which inputs are most important and what triggers them would be a fruitful area of research but may require radical approaches to rank the potential feedbacks and how they affect feeding in poultry.

Neural and endocrine signalling from the periphery to the feeding centre are discussed which seem to have relatively strong evidence. Thyroid hormones and corticosterone are classical endocrine factors related to metabolism. Evidence for the effect of the thyroid hormone T3 on the expression of *AGRP* and *POMC* comes late stage embryos or young chicks with a striking reduction in *POMC* expression in the hatched chick (Byerly et al., 2009). Plasma T3 concentrations are reduced when feeding is restricted (Newcombe et al., 1992) which seems logical in respect of its effects on the hatched chick. Although the authors suggest the effect is not a direct effect of T3 on the *POMC* gene (Byerly et al., 2009). Thyroid hormones and corticosterone as well as the function of genes that favour thyroid hormone production may be reduced with selection for growth to favour anabolism (Vaccaro et al., 2022) and AMPK may be involved in thyroid hormone effects (Lamberigts et al., 2021).

Signalling from the gastro intestinal tract is important in determining overall feed intake. Some of the effects are paracrine effects within the gastro-intestinal tract or the pancreas while others may act over a longer range to alter the brain's response. Although it is difficult to separate effects transmitted by the vagus from directly to the brain. The gastrin/cholecystokinin (GAST/CCK) family are known to regulate appetite and digestive activity. CCK acts as a satiety hormone in the chicken and releases bile from the gall bladder (Dunn et al., 2013a, Covasa and Forbes, 1994, Dimaline and Lee, 1990). In chickens as in mammals CCK probably release pancreatic enzymes and CCK and gastrin alters gastric motility (Choi et al., 1994, Martinez et al., 1993a). *CCK* expression is relatively widespread compared to GAST, which was found at the gizzard-duodenum boundary (Martinez et al., 1993b, Reid and Dunn, 2018). The presence of *CCKAR* expression along with CCK induced contraction suggests CCK alters crop emptying which may act to control food intake (Jiao et al., 2022). Although Peptide YY (PYY) expression is present in the intestine, the pancreas has the highest expression in chickens as of course does pancreatic polypeptide Y (PPY) and PYY is altered by nutritional status (Reid et al., 2017, Gao et al., 2017). From receptor distribution the likely sites of action are the pancreas itself and where by analogy it may release insulin in birds, the duodenum and the brain including the hypothalamus (Gao et al., 2017).

Whilst these factors are likely to have central effects on the POMC/AGRP neurones it is probably through intermediary neurones or brain centres that transmit information from ascending signals from the vagus. However, insulin receptor expression is co-located in POMC neurones (Shiraishi et al., 2011) and injections of insulin increased anorectic POMC expression (Honda et al., 2007) and its effect on feeding is through the melanocortin system (Shiraishi et al., 2008). There are some reasons to speculate that the avian pancreas in its classic role of secreting insulin and glucagon alongside its role as a target and producer of known satiety factors such as PYY and the receptor for CCK may have greater importance in birds than mammals in the control of feed intake and metabolism (Dupont et al., 2022).

Many other peptides and systems exist which are likely to affect feedback to the POMC and AGRP neurones to control feeding behaviour and metabolism, but conclusive proof for many may only beginning to be established or remain in dispute for poultry. Many of these systems have strong cases for involvement, including a number of adipokines (Estienne et al., 2021, Diot et al., 2015), specific nutritional related molecules (Wang et al., 2014), and bile acids (Piekarski et al., 2016). Because it is clear that birds peripheral response to changes of feeding and metabolism are not the same as mammals there are considerable opportunities for carefully controlled scientific endeavours to investigate these systems and to understand precisely how they work. All these systems are likely to have important effects feed intake and its ultimate effect on growth and body weight which have commercial importance.

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Protein nutrition

Impact of Insect-Based Diets on Digestibility, Performance and Product Quality (M. Gariglio)

Marta GARIGLIO

Dept. of Veterinary Sciences, University of Turin, Italy
Corresponding author: marta.gariglio@unito.it

Abbreviated Title: Insect digestibility in poultry

Summary

In a context of increasing world population and poultry meat demand, the research of new raw materials for feed production is needed. Insects derived proteins could be one of the solutions. Nowadays, seven insects' species are authorized in Europe for poultry feeding (Commission Regulation (EU) 2021/1372 of 17 August 2021), with *Hermetia illucens* and *Tenebrio molitor* meals as the most promising. However, digestibility studies of insects' meals are required, in order to determine the feasibility of the inclusion of these ingredients in poultry diet. Digestibility studies aim at evaluating the ingredient retention of nutrients by the birds and, on the other hand, how a complex diet containing the test ingredient is digested. Despite the great potential of using insects in poultry species, few works evaluated their digestibility, and the obtained results vary considerably. For these reasons, further research is needed in order to expand the knowledge on this field.

Key words: insect, digestibility, poultry

Text

In the next decades a continuous increase in poultry meat demand for human consumption is expected (Alexandratos and Bruinsma, 2012). The 92% of poultry meat production comes from specialized broiler systems where the diets are mainly constituted by cereal grains and oilseed cakes and having a high environmental impact (in terms of land, energy and water uses) (Mottet and Tempio, 2017). In this context, the use of insects as substitutes for traditional protein sources in feed is recognized as one of the potential solutions, helping in the achievement of a more sustainable production systems (Onsongo *et al.*, 2018). Indeed, insects are rich in valuable nutrients (proteins, amino acids, fat and energy, vitamins, minerals, and bioactive substances), and have a lower environmental impact than other protein sources (Gasco et al., 2019). Several insect species can be efficiently raised on non-edible resources for human and farm animals, with a lower environmental impact in terms of water and land use compared to the traditionally used protein sources (Ojha *et al.*, 2020). Recently, the European Union has authorized seven insects' species for poultry feed (Commission Regulation (EU) 2021/1372 of 17 August 2021):

Hermetia illucens (black soldier fly), *Musca domestica* (common housefly), *Tenebrio molitor* (yellow mealworm), *Alphitobius diaperinus* (lesser mealworm), *Acheta domesticus* (house cricket), *Gryllodes sigillatus* (banded cricket), and *Gryllus assimilis* (field cricket). One of the most promising insect species that could be used in poultry feeding is the *H. illucens* (HI), a Diptera from Stratiomyidae family. Another promising insect species is the *T. molitor* (TM), a Coleoptera of the Tenebrionidae family. Dried larvae meal of these two species can be provided as it or it can be further processed to obtain oil-extracted meal (partially or totally defatted) (Dörper *et al.*, 2021). However, in order to determine the feasibility of the dietary inclusion of insects' meal in poultry, digestibility studies should be conducted. Ingested feeds are not completely absorbed by the animal: part of the ingested feed is not used, passing through the digestive tract, and being excreted in the droppings. Several factors may affect nutrient digestibility, such as animal (species, strain, age), the ingredient chemical composition, and it is also affected by technological treatments (thermal, chemical and/or mechanical). The *in vivo* digestibility studies are useful to understand the amount of nutrients (organic matter, amino acids, fat and metabolizable energy) that are absorbed by the digestive tract and used for metabolism (Atchade *et al.*, 2019). In order to determine the amount of nutrients that are retained by the body, the use of an inert marker (indigestible by the bird, i.e. Titanium oxide or Celite) is necessary, including it in the experimental diet (from 0.3 to 1% on feed basis depending on the marker). Thus, for this purpose, two main digestibility studies can be conducted with different methodologies: ingredient digestibility and diet digestibility. The ingredient digestibility is usually the first step, including the test ingredient in a simple, basal diet at a known percentage (usually at the 25% of inclusion). The ingredient digestibility study take place for four consecutive days, after an adaptation period, with excreta collected and weighed daily (De Marco *et al.*, 2015). Thus, together with the dry matter (DM), organic matter (OM), crude protein (CP), and ether extract (EE) digestibility, it is possible to estimate the metabolizable energy value of the ingredient, fundamental for the formulation of a complex diet including the test ingredient. Indeed, the second step of a digestibility study is represented by the diet digestibility, where the test ingredient is included in a more complex diet formulating to be similar to a commercial diet, helping in the comprehension of the possible interactions between the test ingredient and the other raw materials included in the diet. In this case, the test ingredient is usually included in a lower percentage compared to the method described above. Also in this case, the excreta are collected daily for four consecutive days after an adaptation period.

Few researches assessed the HI and TM apparent nutrient digestibility and metabolizable energy values for poultry (Table 1).

Table 1. Summary of apparent nutrient digestibility and metabolizable energy values of HI and TM larvae meal.

Type of insect meal tested	**DM%**	**OM%**	**CP%**	**Lys %**	**Met %**	**EE%**	**AME (Mj/kg)**	**AMEn (Mj/kg)**	**TMEn (Mj/kg)**	**Author**
HI, full fat	53	66	51	46	42	99	17.38	16.60	-	De Marco *et al.* (2015)
TM, full fat	60	66	60	85	80	88	16.86	16.02	-	
HI, partially defatted	-	-	-	88.5	91.68	-	-	-	14.91	Matin *et al.* (2021)
TM, full fat	-	-	-	91.56 ± 0.81	89.82 ± 1.55	-	-	-	21.77 ± 0.44	
HI, highly defatted	75.8 ± 1.13	-	47 ± 2.61	83.9	85.3	93.1 ± 0.64	13.77 ± 0.48;	12.15 ± 0.42	-	Mwaniki and Kiarie (2019)
HI, partially defatted	63	69	62	80	83	98	16.25	11.55	-	Schiavone *et al.* (2017)
HI, highly defatted	59	64	62	80	78	93	14.87	9.87	-	

HI: *H. illucens*; TM: *T. molitor*; DM: dry matter; OM: organic matter; CP: crude protein; Lys: lysine; Met: methionine; EE: ether extract; AME: apparent metabolizable energy; AMEn: apparent metabolizable energy corrected to zero nitrogen balance; TMEn: true metabolizable energy corrected to zero nitrogen balance.

From the literature review, it can be observed that the apparent digestibility and energy values obtained for HI vary considerably (De Marco *et al.*, 2015; Schiavone *et al.* 2017; Mwaniki and Kiarie, 2019; Matin *et al.* 2021). This could be due to the different type of HI used, that presented different amount of fat (full fat, partially or highly defatted). Moreover, also the chemical or mechanical treatments used for fat extraction from the HI meal may affect its composition and, consequently, the nutrient and energy absorption and digestibility. On the other hand, only two studies evaluated the apparent digestibility and energy values vales of the TM meal in poultry (De Marco *et al.*, 2015; Matin *et al.* 2021).

On the other hand, few studies evaluated the digestibility of a diet including insects' meal in poultry. The most recent studies are reported below, evaluating the dietary inclusion of HI meal (Table 2).

In general, when considering the digestibility of the HI in a complex diet, the results may vary considerably depending on the poultry species considered and on the amount of HI inclusion.

In conclusion, digestibility studies result to be the first important step when considering the possibility to dietary include insect meal in poultry. However, due to the high results variability (in terms of nutrient digestibility), further research is needed in order to better understand how insect meal can be digested and absorbed by poultry.

Table 2. Effects of the dietary HI meal inclusion in poultry on diet digestibility (adapted from Gasco et al., 2019).

Author	**Avian species**	**Insect form**	**Inclusion**	**Results**
Kim *et al.*, 2021	Broiler chickens	Defatted larva meal	6.5-13%	Decrease in apparent ileal digestibility for CP.
Cullere *et al.*, 2016	Broiler quails	Defatted larva meal	10-15%	Higher EE digestibility in 10% group than other groups.
Gariglio *et al.*, 2019	Broiler ducks	Defatted larva meal	3-6-9%	Lower CP digestibility in the starter period for 9% HI than other diets. Higher EE digestibility in grower and finisher.
Bovera *et al.*, 2018	Laying hens	Partially defatted larva meal	7.3-14.6%	Decrease in apparent ileal digestibility of DM, OM and CP.
Cutrignelli *et al.*, 2018		Highly defatted larva meal	17%	Decrease in apparent ileal digestibility of DM, OM and CP.
Navasero et al., 2022		Full fat larva meal	3%	Higher CP and GE digestibility.

DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract, GE: gross energy.

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Small scale family poultry farming

Making family poultry value chain more mature and sustainable at a small scale (F. Gueye)

Fallou GUEYE

(1) FAO Representation to Djibouti & Intergovernmental Authority on Development (IGAD), 6 Rue de Venise, B.P. 2588, Djibouti, Republic of Djibouti

Corresponding author: efgueye@gmail.com

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Summary

Family poultry (FP), which make up more than 80% of the world's poultry stocks and kept in small numbers ranging from as few as one up to about 20, are critically important in low- and lower middle-income countries (LLMICs) of Africa, Asia, Latin America and the Pacific. FP value chain plays key roles in food and nutrition security, income generation, livelihoods and conservation of indigenous breeds. Analysing of grey literature and mid-term reports of targets and indicators towards achieving 17 Sustainable Development Goals (SDGs) by 2030 showed that FP are central to contribute to achieving directly 12 of the SDGs. The growing demand for poultry products in LLMICs, driven by population growth, higher incomes, scarcity of land and urbanization, represents a huge opportunity for hundreds of millions of poor smallholder poultry farmers, processors and marketers, many of whom are women, to meet that market demand and rise out of poverty. Improving the efficiency of poultry production in LLMICs, especially the productivity per poultry bird, can triple poultry productivity while reducing by more than a third its adverse environmental impacts, including reducing emissions of greenhouse gases (GHG), in those LLMICs that are less GHG emitters but highly negatively impacted by them. However, FP are facing many constraints, including high mortality (mainly due to Newcastle and other avian diseases). Significant improvements in FP value chain can be achieved through well-designed and implemented research and development programmes that endow FP actors with necessary knowledge and skills. In addition to the need for substantial improvement in human and institutional capacity building, planners and policy makers must be sensitized about the potential of poultry as a tool in poverty reduction, food security and gender equity strategies. The paper explores strategies and options to make small-scale FP value chain more mature and sustainable.

Keywords: Family poultry, mature poultry, small-scale farming, sustainable poultry, value chain

Introduction

The growth of the world human population, which is expected to increase from 7,795 million in 2000 to 9,735 million in the year 2050 (United Nations 2022), will take place largely in low- and lower middle-income countries (LLMICs) of Africa, Asia, Latin America and the Caribbean, and the Pacific. According to the World Bank (2022), low- and lower middle-income countries LLMICs) are nations that have a per capita gross national income (GNI) of less than US$ 1,026 or between US$1,026 and US$3,995. In June 2021, 82 nations were classified as LLMICs (46 in Africa, 23 in Asia, 6 in Oceania, 4 in Central America, 2 in Latin America and the Caribbean and 1 in Europe). Most of the 720 to 811 million people still suffering from hunger in 2020 (FAO et al. 2021.) live in LLMICs, especially in the arid zones of Africa and Asia. Poultry production represents one of the alternatives to feed the fast-growing human population. According to data from the United Nations Food and Agriculture Organization (FAO), the total poultry population in the world (chickens, ducks, geese, guinea fowls and turkeys) is about 27.9 billion heads in 2019, with the largest share being chickens (about 93 percent). The number of chickens worldwide has more than doubled since 1990. The number of chickens, which was 14.38 billion in 2000, reached 25.9 billion in 2019, with the largest share of chicken population kept in Asia (15.8 billion heads), followed by the Americas (5.8 billion), Africa (2.0 billion) and Europe (2.0 billion). However, according to Branckaert and Guèye (2000) and Guèye (2003a), most of the conditions required by the (semi-) industrial poultry sub-sector are not met in LLMICs, namely (i) the ability to purchase most inputs, i.e. improved birds, feeds, vaccines, drugs and equipment; (ii) the availability of a highly skilled manpower; (iii) the presence of a strict disease control; and (iv) the existence of national domestic markets able to absorb poultry products at attractive prices by consumers with adequate purchasing power. In fact, prior to developing medium to large-scale poultry units, either for broiler or egg production, it is important to achieve either self-sufficiency in cereal products or to generate the necessary hard currencies provided by the export of oil or other expensive raw materials, or to have a developed services sector (Guèye 2003a). Poultry and people have had a shared history for thousands of years and current trends suggest that this is not about to change in the near future.

Family poultry (FP), which comprises extensive and intensive small-scale poultry production, is still important in LLMICs of Africa, Asia, Latin America and the Pacific (Sonaiya et al. 1999, Figueiredo 2012, Guèye 2012, Alders et al. 2018, 2022). As the challenges and opportunities for small-scale FP production are reviewed, it is crucial to bear in mind that poultry are frequently an essential part of the fabric of human societies, especially in in LLMICs. While making one of the best uses of available natural resources, FP constitute an important component of the agricultural and household economy in LLMICs, a contribution that goes beyond direct food production for the fast-growing human population as well as employment and income generation for resource-poor small farmers, especially women. They also serve as a means of capital accumulation and as a barter product in societies where there is no circulation of currency. Furthermore, they are closely linked to the religious and socio-cultural lives of several million resource-poor farmers for whom poultry ownership ensures varying degrees of sustainable farming and economic stability by minimizing risks and strengthening the cohesion within traditional communities. Despite its significant contribution to poverty alleviation, food security and the well-being of the human population, especially in disabled and disadvantaged groups in less-favoured areas of LLMICs, FP does not receive due attention from many agricultural policy makers (including livestock specialists). Small-scale FP poultry is not yet regarded by many researchers, development and extension workers as an area of importance in terms of political aspects and scientific prestige. The paper explores strategies and options to make small-scale FP value chain more mature and sustainable.

Family poultry production systems and significance

Production systems

Typically, four family poultry (FP) production systems can be distinguished in LLMICs (Sonaiya et al. 1999, Branckaert and Guèye 2000, Guèye 2002, 2003a, FAO 2004, Alders et al. 2018, FAO 2022), namely (Table 1)

(1) the small extensive scavenging system: flock size of 1-5 adult birds of local breed, scavenging, no regular water or feed, little or poor night shelter;

(2) the extensive scavenging system: flock size of 5-50 adult birds of local or crossbred breed, regular water, supplementary feeding, improved shelter, care of chicks in the first weeks, vaccination against Newcastle disease and other diseases (e.g. fowl pox, fowl cholera, Gumboro disease, coccidiosis), when necessary, and treatment for parasites;

(3) the semi-intensified system: flock size of 50-200 adult birds of commercial or crossbred or local breed, as in 2. above, with genetically improved breeds and balanced diets; and (4) the small-scale intensified system: flock size of 50-900 broilers or 30-500 layers of commercial breed, as in 3. above, with further improvements in overall husbandry conditions.

Criteria*	**Small extensive scavenging**	**Extensive scavenging**	**Semi intensified**	**Small-scale intensified**
Production/farming system	Mixed, poultry and crops, often landless	Mixed, livestock and crops Sometimes small land	Usually poultry only	Poultry only
Other livestock raised	Rarely	Usually	Sometimes	No
Flock size (adult birds)	1-5	5-50	50-200	50 - 900 broilers** 30 - 500 layers

Poultry breeds	Local	Local or crossbred	Commercial or crossbred or local	Commercial
Stable access to affordable inputs and services required for market oriented intensive production and markets capable of selling large numbers of birds or poultry products at a sustainable price in a single day	No	No or rarely	Most of the time	Yes
Source of new chicks	Natural incubation	Natural incubation	Commercial day-old chicks or rarely natural incubation	Commercial day-old chicks or pullets or artificial incubation
Feed source	Scavenging; almost no supplementation	Scavenging; occasional supplementation	Limited scavenging; regular supplementation	Commercial balanced ration or "self-made» balanced ration
Drinking water	Almost no dedicated supply	Occasional supply	Regular supply	Regular supply
Poultry housing	Seldom; usually made from local materials or kept in owner's house Overnight	Sometimes; usually made from local materials	Yes; conventional materials; houses of variable quality	Yes; conventional materials; good quality houses
Access to veterinary services and veterinary pharmaceuticals	Rarely	Sometimes	Yes	Yes
Mortality (without any vaccination)	Very high >70%	Very high >70%	Very high >70%	Very high >70%

Mortality (with effective vaccination against endemic vaccine-preventable diseases)	Medium <30%	Medium <30%	Low to medium <20%	Low <10%
Biosecurity risk and management strategy	Medium: Community biosecurity strategy required; rate of spread of disease within flocks usually lower than intensive systems	Medium: Community biosecurity strategy required; rate of spread of disease within flocks usually lower than intensive systems	High: Community and individual unit biosecurity strategy required; rate of spread of disease within flocks usually somewhat lower than intensive systems	High: Individual poultry unit biosecurity strategy required; rate of spread of disease within intensive units usually high
Access to reliable electricity supply	No	No	Most of the time/ renewable energy	Yes/ renewable energy
Existence of conventional cold chain	No	Rarely	Yes/rarely	Yes
Access to urban markets	Rarely	Rarely or indirect	Most of the time	Yes
Products	Live birds, meat	Live birds, meat, eggs	Live birds, meat, eggs	Specialized on a single product: live birds, meat or eggs
Time devoted each day to poultry management	<30 minutes	<1 hour	>1 hour	>1 hour

* This classification of family poultry production systems is a guideline and not intended to be prescriptive.
** Can be as high as 2,000 in some countries.

All of these systems are encountered in FP, although in the very few cases in which the intensive management system is practised, it is the small-scale option that is adopted. The choice of system is largely determined by the availability of resources and inputs, i.e. housing, cages, feed, drugs and time/attention (Guèye 2002, 2003a, FAO 2004) (Figure 1). Thus, most FP-keeping producers adopt the small extensive scavenging system, extensive scavenging system, semi-intensified system and small-scale intensified system, in that order. Also, these management systems frequently overlap; thus, small extensive scavenging is sometimes coupled with feed supplementation, extensive scavenging with night confinement but without feeding; or standard poultry cages in confined space. Under the extensive poultry management systems, there is almost no health care. Nevertheless, according to Guèye (1999), 35–79% of resource-poor village poultry farmers in Africa rely on ethnoveterinary medicine which is mainly based on the use of natural products, especially locally available plant products. Thus, FP in LLMICs are often maintained with very low levels of inputs (i.e. land, labour and capital), though there are possible variations between and within regions or countries. The level of inputs also depends on the keeper's or household's socio-economic circumstances. On the whole, FP can be managed by even the poorer social strata of the local communities.

In many LLMICs across Africa and Asia, it has been estimated that more than 80% of the poultry population is found in family-based poultry production systems, contributing 60-95% of poultry products and 20-32% of total animal protein intake (Branckaert and Guèye 2000, Guèye 2003a, Besbes et al. 2012, Pym et al. 2006, de Bruyn et al. 2015, Wong et al. 2017, Alders et al. 2022). All over the developing world these low input–low output poultry husbandry systems have been a traditional and integrated component of rural, many peri-urban and some urban households or small farms, and are likely to continue in the foreseeable future.

Flock characteristics

According to Guèye (2003b), household poultry flock size ranges from 1 to 95 in Africa, from 5 to 35 in South America, and from 30 to 2000 in Asia. Foundation stock is usually obtained from the market or through exchange (barter) or as gifts. Flock size is associated with the purposes for keeping poultry (e.g. consumption, income, gifts, socio-cultural and/or religious ceremonies, ornamental poultry, hobby, gifts, security and breeding stock). Under the free-range and backyard systems, the female to male ratios (or sex ratios) in flocks amount to 1:1–8:1 for chickens or domestic fowls, 2:1–5:1 for guinea fowls, 0.7:1–1.7:1 for Muscovy ducks (the duck species mostly kept in dry areas, especially in Africa and Asia), 1.1:1–2.7:1 for turkeys, and l:1 for pigeons (pigeons are mostly kept in pairs). The numbers of males tend to be lower in flocks with high proportions of growers, and to be higher in larger flocks. Males are generally removed from the flocks at an early age in relation to the objectives of the poultry enterprise. In FP husbandry systems, various species of poultry (i.e. chickens, ducks, guinea fowls, geese, pigeons) are kept, albeit chickens largely dominate family flock composition. Ducks are popular in Asia and South America. Birds are of indigenous or local types, exotic breeds and crosses between two types of poultry breeds. Under extensive management systems, FP usually scavenge in and around the compound of household, feeding on locally available resources e.g. earthworms, household refuses, insects, residues from the harvest. Their feed is sometimes supplemented with agricultural by-products, especially for laying birds and poultry intended for sale. FP is usually raised together with other domestic animals (e.g. other monogastric species such as pigs and rabbits, small and large ruminants) and in some cases with fish. Animal agriculture is often integrated into crop cultivation and associated with off-farm activities. On the whole, integrative farming systems have always promoted sustainable agricultural production on small farms (Guèye, 2002, Besbes et al. 2012, de Bruyn et al. 2015, Alders et al. 2018).

Productivity

Productivity of poultry birds depends on the management system adopted (Guèye 2003a, FAO 2004, Besbes et al. 2012). Figure 2 illustrates the effect of the level of intensification on the productivity of chickens. According to Guèye (2003b), under the small-scale extensive scavenging system systems, a guinea fowl produces 37–95 eggs per year, a Muscovy duck 30–80 eggs per year, a goose 20–40 eggs per year, a turkey 25–100 eggs per year and a pigeon 14–29 eggs per year. The average egg weight amounts to 30–65g for domestic fowl hens, 30–40g for guinea fowls, 50–85g for Muscovy ducks, 65–95g for geese, 80–100g for turkeys and 10–20g for pigeons. Sexual maturity of local female poultry occurs late, at 24–36 weeks in domestic fowl hens, 28–42 weeks in guinea fowl hens, 28–32 weeks in Muscovy ducks, 35–47 weeks in geese, 20–48 weeks in turkeys and 17–19 weeks in pigeons. Hatchability is usually high in domestic fowls (60–95%), Muscovy ducks (55–90%), turkeys (60–95%), pigeons (70–95%), but much lower in guinea fowls (20–50%). Because of poor hatching rates observed in guinea fowl hens, chickens and ducks are used in many local communities for hatching guinea fowl eggs and brooding guinea fowl keets. A domestic fowl hen can hatch 15–20 guinea fowl eggs, and a Muscovy duck can sit on 35–40 guinea fowl eggs. Indigenous or local poultry grow slowly and are rather small. According to Guèye (2003b), adult female chickens weigh 0.7–2.2 kg and male chickens weigh 1.2–3.2 kg; adult guinea fowls weigh 1.7–2.0 kg (female) and 2.0–2.5 kg (male); adult Muscovy ducks 2.8–3.5 kg (female) and 3.5–5.0 kg (male); adult female geese weigh 2.5–4.0 kg and males weigh 3.8–6.2 kg; adult female turkeys weigh 5.5–7.5 kg and males weigh 8.5–13.0 kg; and adult pigeons weigh 0.24–0.30 kg (female) and 0.29–0.45 kg (male). However, meat and eggs from indigenous stocks are more esteemed by LLMICs' consumers, in comparison with those from intensively raised poultry flocks. Consumers generally argue that products from indigenous poultry are tastier and healthier (because synthetic drugs, such as antibiotics, are rarely used); therefore, they often fetch premium prices. Because of its low productivity, FP in LLMICs has been undervalued and is frequently considered by farmers as an insignificant occupation compared with other agricultural or trade activities. As a result, it does not receive due attention from many agricultural policy makers (including livestock specialists). Nevertheless, FP is a valuable asset to local populations because it is not only a source of income, food but is also critical to strong socio-cultural linkages in LLMICs, especially in disadvantaged groups and less-favoured areas.

Significance of poultry

The keeping of poultry by local communities has been practised for many generations in LLMICs. More than 85% of rural families in sub-Saharan Africa, more than 90% of tribal families from 35 surveyed villages in the five districts of western India, 92% of the 100 surveyed respondents in Madhya Pradesh in central India, 89% of the rural households in Bangladesh and 90–95% of the households in rural Cambodia keep one or more poultry species (Guèye 2003a, 2005). All ethnic groups tend to be involved in FP production (Guèye 2002, 2005), and birds are kept for many reasons (Guèye 2003a, 2003b, Alders et al. 2018). Poultry keeping has a symbolic importance within the context of many social and cultural activities (e.g. special banquets for distinguished guests, gifts, cocks as alarm clocks for the villagers) and/or religious ceremonies (e.g. cocks as offerings to the deities). Furthermore, the major role played by women, assisted in some cases by children, in FP production in LLMICs is widely recognized (Sonaiya et al. 1999, Branckaert and Guèye 2000, Guèye 2003a, 2003b, 2005, FAO 2004, de Bruyn et al. 2015, Alders et al. 2018, 189 2022). For example, more than 70% of chicken owners in rural areas of sub-Saharan Africa are women, while traditionally pigeons belong only to children (boys) (Guèye 2003a).

In LLMICs, keeping poultry is in many cases considered as the first step in animal-rearing activities, especially after events such as climatic disasters (e.g. droughts, cyclones, hurricanes and floods), civil wars, political and economical instabilities lead often to drastic decrease in numbers of livestock (i.e. goats and cattle). This was, for example, the case in Mozambique, after as a long-lasting war and drought. For example, selling 4–5 chickens enabled rural women in Mozambique to get access to a goat (Bagnol 2001). Furthermore, keeping poultry for smallholder farmers represents a household savings, investment and insurance as the value of the birds increases over time. Under traditional management systems, most eggs from various poultry species are allowed to incubate under the mothers-poultry, because FP-keeping farmers in LLMICs are aware of high mortality, especially in growing birds in rural areas. Keeping even a small flock is their major concern partly because of the social, cultural and religious importance of poultry. Mortality rates in chickens are estimated at 53% up to four weeks of age in sub-Saharan Africa, 35–40% over the whole rearing period in Bangladesh and 40% in chicks in Western India (Guèye, 2003a).

Poultry contributions to Sustainable Development Goals

In 2015, all Member States of the United Nations signed up to achieving the Sustainable Development Goals (SDGs) by 2030. The FAO is committed to poultry production and, through the International Network for Family Poultry Development (INFPD) and other initiatives such as the promotion of agroecological poultry production. The importance of poultry for poverty reduction, food security, and the empowerment of women and youth is highlighted. Poultry production, the largest livestock sector, accounts for more than 30% of all animal protein and makes a major contribution to national economies worldwide. The growing demand for poultry products in developing countries, driven by population growth, higher incomes, scarcity of land and urbanization, represents a huge opportunity for hundreds of millions of poor smallholder poultry farmers, processors and marketers, many of whom are women, to meet that market demand and rise out of poverty. The increasing demand for food required to meet the nutritional needs of a growing global population and the challenges facing agriculture now and into the future are among our most important challenges in the 21st century (Wong et al. 2017). Globally, up to 1.5 billion people are employed indifferent poultry product value chains. Poultry represents an important sector in animal production, with backyard flocks representing a huge majority, especially in the developing countries. In these countries, villagers raise poultry to meet household food demands and as additional sources of incomes. Poultry production can boost economic growth through direct contribution to rural livelihoods and agricultural output, and, considering the sector's various linkages with other industries, through the multiplier effects of poultry products along the value chains (FAO 2018). Policies and strategies that promote appropriate sustainable poultry systems lead to improved animal production and welfare, higher labor productivity, and value-adding to production (Alders et al. 2021). Well-managed poultry production systems are central to contribute to achieving directly 12 of the 17 SDGs, especially in countries with a long history of animal production (IFAD 2020). Sustainable poultry production contributes indirectly to SDG11, SDG16 229 & SDG17.

Table 2 summarises the potential direct contributions of FP production systems to twelve of the 17 SDGs. By coping with major health-related constraints to FP production efficiently and sustainably, their potential contributions to sustainable development can be strengthened.

Table 2. Contributions of four priority areas for poultry in achieving the SDGs (UNDP 2015, Wong et al. 2017, FAO 2018, 2022, IFAD 2020, Alders et al. 2021).

Priority areas for poultry	**Sustainable Development Goal**
① **Poultry and decent work and sustainable economic growth and industry and innovation and infrastructure**	
· Generating income in support to households' expenses. · Connecting poor poultry-keeping farmers to markets. · Doubling the productivity of poor smallholders' poultry through better feeding, veterinary care and breeding. · Developing 'Decision tools for family poultry development' to support effective investment planning to optimize poultry's contribution to economic growth. · Developing new vaccines against Newcastle disease, which costed e.g. poultry producers in California, U.S.A around US$162 million in 2002- 2003 resulted from more than 2,500 premises depopulated (4 million birds). · Poultry product processing is an emerging and fast-growing industry in developing countries.	Goal 1: End poverty in all its forms everywhere Goal 8: Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all Goal 9: Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation
② **Poultry and equitable livelihoods**	
· Applying gender-transformative approaches that give women in poultry raising, processing and trading greater access to, and control over, poultry resources. · Developing labour-saving technologies for poultry feeding. · Insuring never-before-insured poultry actors against impactful viral diseases and other losses affecting the poultry industry. · Developing options to reduce barriers to safe and sustainable domestic and regional trade in poultry products.	Goal 5: Achieve gender equality and empower all women and girls Goal 10: Reduce inequality within and among countries
③ **Poultry and food security and safe and healthy balanced diets, including animal-sourced foods**	

· Doubling the supply of poultry-sourced foods through better feeding, breeding and health · Reducing antimicrobial resistance through judicious use of antimicrobial drugs in poultry. · Reducing the burden of zoonotic diseases through better poultry health and promotion of ‘one health’ approaches that integrate veterinary, medical and environmental understanding. · Improving food safety in informal markets (where most poultry-sourced foods are traded in developing countries); poultry account for more than 30% of all animal protein. · Improving children’s cognitive development through poultry-sourced food.	Goal 2: End hunger, achieve food security and improved nutrition and promote sustainable agriculture Goal 3: Ensure healthy lives and promote well-being for all at all ages Goal 4: Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all
④ **Poultry and sustainable ecosystems**	
· Contributing to the provision of clean, renewable energy through manure for biogas. · Making more efficient use of water resources by improving forage varieties and poultry feeding regimes.	Goal 7: Ensure access to affordable, reliable, sustainable and modern energy for all
· Measuring (for the first time) and significantly reducing greenhouse gas emissions from small-scale poultry systems in developing regions; poultry meat and eggs contribute around 8% of sector’s Greenhouse Gas (GHG) emissions. · Supporting sustainable feed resource base management, control of predators and soil fertility. · Providing better returns for producers and better nutrition for all.	Goal 12: Ensure sustainable consumption and production patterns Goal 13: Take urgent action to combat climate change and its impacts Goal 15: Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss

Strategies and options for mature and sustainable small-scale family poultry value chain

Science, innovation and technology needs

If science, innovation and technologies are to contribute to the improvement of family poultry (FP) value chain, a wide range of actors playing roles in FP value chains and should be considered (Figure 3). FP services include a range of inputs, including research, training, veterinary, extension, farmers’ organization, financial and advisory services. Mature and sustainable small-scale FP value chains are those that are built on current practices and capabilities of actors. Additionally, they should make efficient use of locally available resources (i.e. farmers' knowledge and practices, feed resources, building materials, equipment). Strategies and options for mature and sustainable small-scale FP value chain in LLMICs should be related to the perceptions, needs, priorities, interests and suggestions of relevant members of local communities, as well their different component groups, i.e. men, women, boys, girls, young and old, poorer families, wealthier families, members of different sociocultural groups. Achieving mature and sustainable small-scale FP value chain requires:

● adopting (i) participatory and inclusive approaches from the outset of any FP project (Guèye 2002, 2003a), (ii) a philosophical approach for all interventions that must be integrated and holistic ('generalistic') by taking into consideration socio-cultural, economic, and environmental dimensions and (iii) a scientific approach that has to be multi and transdisciplinary because most farmers in LLMICs, especially women, undertake a wide range of on-farm and off-farm activities (Guèye 2002, 2003a, 2003b);

● promoting consensual and joint actions through multi-stakeholder and intersectoral dialogue where all actors are equally considered, especially women and men from vulnerable groups (Alders et al. 2022);

● identifying or developing and promoting the adoption of good practices tested and validated under similar resource-poor environments (including management of endemic poultry diseases, good feeding practices, sound animal welfare, promotion of responsible consumption and equitable income generation) to make the FP value chain more mature, resilient and environmentally sustainable (Guèye 2002, Alders et al. 2022);

● build capacities of actors in support of sustainable good practices;

● providing enabling environments and conditions, including by developing and enhancing policies and legal frameworks that foster sustainable FP value chain. This requires investment in the value chain and creating incentives for good practices and FP producers.

Organizational, technical and financial assistance needs

The formation of farmers' cooperatives, whenever possible, will facilitate their training and education with a view to enhancing their skills. The supply of inputs (i.e. feed supplementation, improved birds, drugs and vaccines) would also be easier because of the economies of scale. The cost of buying drugs or vaccines can be shared among several FP-keeping farmers and the marketing of poultry products may be pooled, thus reducing transportation costs. Furthermore, genetic improvement could take place through cock exchanges among cooperative members. Finally, cooperatives would attract technical assistance (e.g. fertile eggs for hatching, veterinary assistance, feeds and credits/loans) that would be virtually impossible for an individual FP-keeping farmer to acquire.

Training and education in FP are difficult and time-consuming tasks (Guèye 2002, 2003a, 2003b). Although it is essential to improve farmers' skills, this must be done gradually. FP producers, especially women in rural areas, tend to be fluent only in local languages. They generally cannot write. In addition to being illiterate, many of them are also innumerate and have no training in management. These aspects should determine training and communication methods to be used. Thus, unconventional (Bagnol 2001, Guèye 2002, 2003a) methods such as theatres, songs and learning-by-doing should be preferred and simple extension messages must be used. Moreover, since women have a great many other activities, training sessions, as well as meetings, must be brief and frequent. They must be scheduled in those periods when women are not involved in other duties, though this is a challenging exercise. Furthermore, although women, and secondly children, should be the main target groups during training sessions (Branckaert and Guèye 2000, Guèye 2002, 2003a, Wong et al. 2017, Alders et al. 2018, 2022), it is recommended that the whole family, or special interest groups, also be trained (Guèye 2002, 2003a, 2003b). Similarly, since men and boys seem to be generally responsible for the construction of shelters, they should ideally be trained in the domain of poultry housing (2003a, 2003b).

Conclusions

Despite efforts to develop the intensive poultry sub-sector, FP are still very important in LLMICs of Africa, Asia, the Near East, Latin America, Europe and the South Pacific. FP is a valuable asset to local populations because it is not only a source of income, food and well-being, but is also critical to strong socio-cultural linkages in LLMICs, especially in disadvantaged groups and less favoured areas. Agricultural policy makers should therefore pay due attention to this very important but generally overlooked mature and sustainable poultry value chain. Appropriate interventions should include the improvement of farmers' skills in all aspects of poultry management by taking socio-cultural, especially gender, issues into account. Indigenous knowledge systems should also not be undervalued, as they are capable of forming a basis for sustainable FP development, which should be backed by well-designed research. Extensive scavenging FP production systems, the most practised husbandry systems in LLMICs, represent the basis on which a mature, sustainable, profitable and well-adapted semi-industrial poultry sub-sector could be progressively developed. As maturity and sustainability assume preservation of natural resources, as well as technical feasibility, economic usability and socio-cultural acceptance, this evolution should be conducted in the most appropriate socio-economic way, taking into account the specific local features and constraints to be overcome. The sensitivity of the gender concept calls for participatory, open minded and flexible development approaches and strategies for FP. Thus, all relevant members of local communities must be involved in the whole process, and their weaknesses in terms of behaviour, perceptions, constraints and attitudes must be identified and taken into account. This enables FP-keeping farmers to bring about appropriate changes in their farm management.

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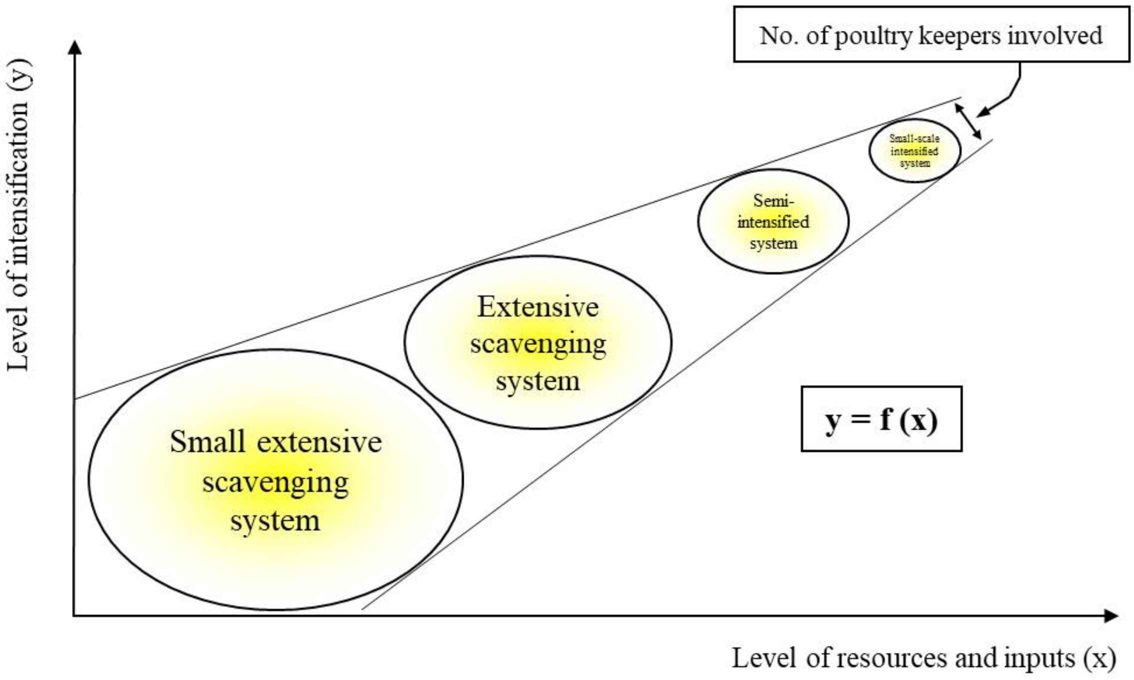

Figure 1. Level of intensification as a function of the level of resources and inputs (Adapted from Guèye 2003a).

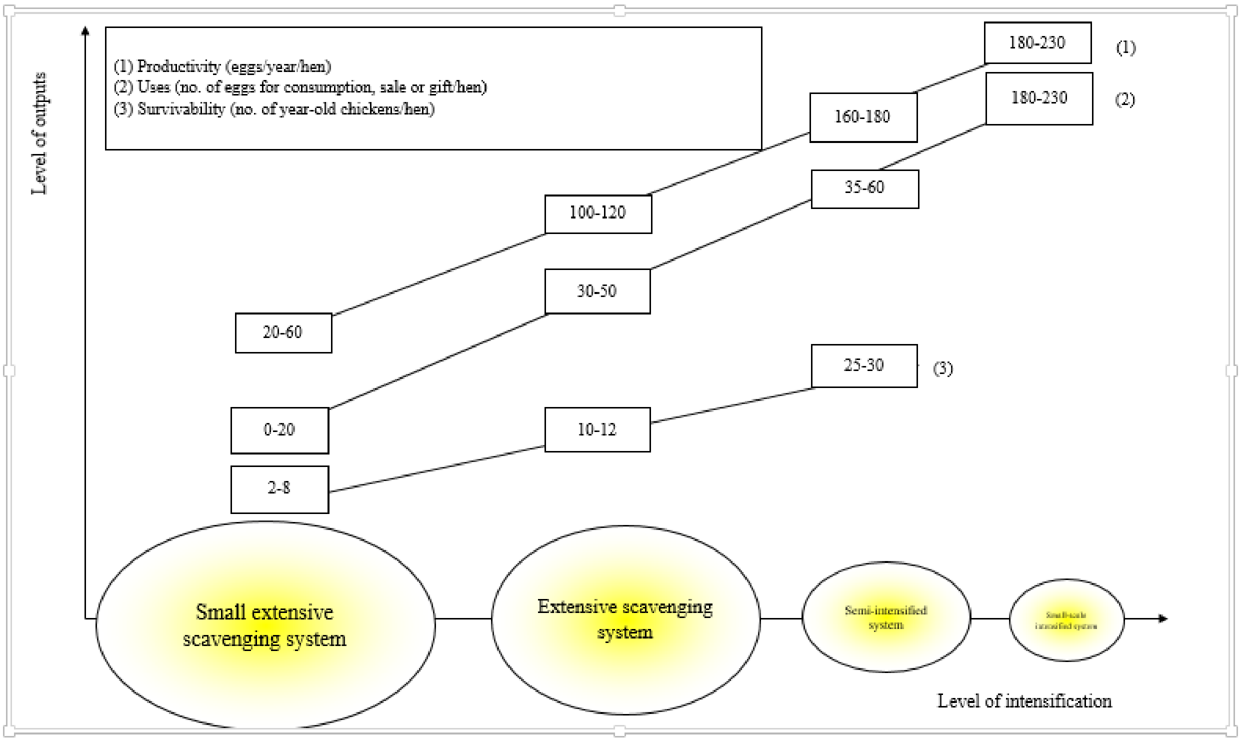

Figure 2. Effect of intensification in production systems on the productivity of chickens (Adapted from Guèye 2003a).

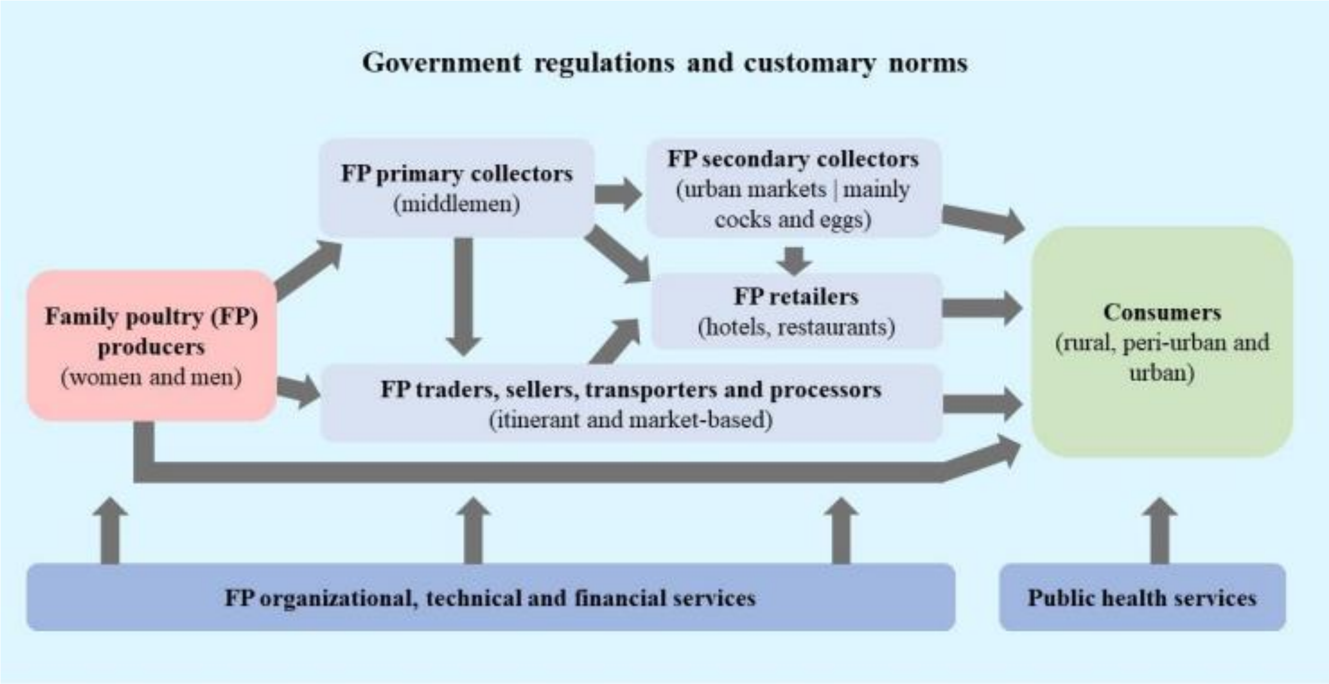

Figure 3. Typical family poultry value chain.

Physiology of perinatal development

Ontogeny and role of the hypothalamo-pituitary-adrenal axis in effects of early-life environment on long-term programming in the chicken (T. Porter)

Tom PORTER

Department of Animal and Avian Sciences, University of Maryland, College Park, MD, USA

Many studies have characterized functional development of the hypothalamo-pituitary-adrenal (HPA) axis in chickens. Early studies, reported nearly five decades ago, demonstrated that experimentally induced stress on embryonic day (e) 14 but not e12 increases plasma levels of the adrenal glucocorticoid corticosterone (CORT), indicating that functional maturation of HPA axis responses to stress occurs on e14 in the chicken. Plasma levels of CORT increase between e10 and e14 in the chicken. Similarly, hypothalamic levels of mRNA for CRH and anterior pituitary levels of mRNA for CRH-receptor I and II and pro-opiomelanocortin (POMC) also increase between e10 and e14, supporting maturation of the hypothalamo-pituitary-adrenal axis by e14. Pituitary growth hormone (GH) production also begins around e14 in the chicken, and GH production can be induced prematurely by treatment with CORT. CORT administration on e11 in ovo increases GH production during embryonic development and results in increased body weight, increased total meat yield, reduced abdominal fat, and improved feed efficiency of the resulting broiler chickens 6 weeks after hatching. Thermal conditioning (TC) during brooding exposes young chicks (3-5 days old) to high ambient temperature for a defined period. This early life exposure imparts long-term resistance to heat stress (HS), allowing birds to survive higher temperatures as they get older. Heat stress on day 28 increases plasma CORT and decreases plasma T3 levels. TC birds have lower plasma CORT levels compared to non-TC birds during the HS period. In addition, during HS, non-TC birds have a higher mortality rate than TC birds. These results indicate that early life TC reduces stress levels and has a protective effect during heat stress. Taken together, these findings indicate that modulation of the HPA axis during embryonic and early post-hatch development has lasting effects on broiler performance.

Key words: growth hormone, corticosterone, embryonic development, growth, heat stress

Feedstuffs

Use of algae in poultry nutrition (E. Coudert)

Edouard COUDERT

E. Coudert (1), E. Baeza (1), C. Berri (1)

(1) INRAE Centre Val de Loire, BOA Unit, 37380 Nouzilly, France **Corresponding author**: edouard.coudert@gmail.com

Abbreviated title: Algae for poultry

Abstract

Nowadays, various parameters currently affect animal productions, especially poultry production. These factors, becoming more and more important for consumers, gather among other things, origin and sustainability of animal feed or impacts on health or environment. Cultivated algae have been integrated to animal production for a long time, especially in fish hatchery and farming. However, algae are less widely integrated in animal nutrition and there is currently a momentum in animal production to include micro- and macro-algae to animal feeds. Algae represents a large amount of potential feed source. These marine plants could present a key role for future poultry production as a new resource income thanks to, for instance, its nutritional composition or richness in polyphenols, polysaccharides, or fatty and amino acids. Various research projects highlighted possible antioxidant, antibacterial, anti-inflammatory effects and/or improved immune system, gut function or metabolism for animal production. Researchers have been working on its capacity to improve poultry performance, nutrition and health for several years now.

Keywords: Algae, chickens, laying hens

Introduction

Nowadays, the concept of natural, organic, and sustainable farming has become more and more important for consumers, especially in developed countries. The farming industry thus has been interested in using natural forms of vitamins and minerals instead of synthetically produced ones. Considering these dynamics, the possibility of using micro and macroalgae as a new source of nutrients and health additives in animal feed formulations has been evaluated. Algae are a very heterogeneous group of plants that can be found in different sizes, shapes, and colors. Thus, the taxonomy linked to this group is complex and controversial in the scientific community. It includes green (Chlorophyceae), brown (Phaeophyceae), and red (Rhodophyceae) algae. Species, habitat, growing conditions (e.g. temperature, light exposure), and collection methods play a key role in the highly variable composition of each type of alga. Algae, as living species, are separated into two main groups: the large macroalgae, also called seaweed, that are often found in the littoral zone, and the small microalgae that are found both on the coast and in the ocean (El Gamal, 2010). Cultivated algae, both micro and macro, have long been incorporated into fish feed, in both hatcheries and farms, and microalgae are considered as a reliable source of nutrients for aquaculture. However, algae are less widely integrated in terrestrial animal nutrition although there is currently momentum to include both micro- and macroalgae in their feed.

Both macro- and microalgae contain various active molecules, such as polyphenols, polysaccharides, lipids, peptides and proteins, and pigments (Harnedy and Fitzgerald, 2011; Michalak and Chojnacka, 2015). Most of the molecules they contain are well characterized and some are known for their antioxidant, antibacterial, or anti-inflammatory properties (Shalaby, 2011; Park, 2015). Even when incorporated at a low level in both aqueous and terrestrial livestock species, algae have been demonstrated to improve animal immune response, lipid metabolism (Nakagawa, 1997; Norambuena et al., 2015), and gut function (Michiels et al., 2012) and to act as antiviral and antibacterial agents (Pulz and Gross, 2004; Becker, 2007; Gouveia et al., 2010). They are also studied as alternative sources of macro- and micronutrients such as proteins, lipids, minerals, amino acids, and n-3 fatty acids, or as a source of specific biological active phytochemicals. In addition to their high protein content, seaweeds are also rich in polysaccharides, polyphenols, and pigments (Korczyński et al., 2015). So far, few studies have examined the use of algae as a major source of macronutrients because of the colossal amount of biomass needed to produce feed. They are, rather, used as supplements, enhancing standard feed formulations to the benefit of animal health or product quality.

This review aims to present the main characteristics of algae used in poultry nutrition and the way they are produced, with a special focus on compounds beneficial for animal health and performance, and product quality, considering both laying hen and broiler chicken production.

Utilization for laying hens’ production

Egg productivity

For a long time, the nutrition of laying hens focused on sustaining egg production with the objective to maintain or improve it in parallel with other traits related to egg quality and animal health. It has been shown that algae can affect egg productivity. Indeed, supplementing 80-week-old Hy-Line Brown layers with 2% of *Chlorella vulgaris* increased egg productivity, from 55.4% in the control to 59% in the supplemented group (Zheng et al., 2011). Supplementing 22- to 36-week-old Lohmann Brown hens with 0.5% of this microalga also increased egg weight (60.4 g vs 58.9 g for the control group), but only at the beginning (1st to 3rd months) and end (6th to 8th months) of the laying period, with no effect on hens’ health or the protein content of eggs (Halle et al., 2009). Use of the red microalga *Porphyridium* sp. has also been tested in 30-week-old White Leghorn hen diets at 5% or 10% for 10 days. These treatments had no effect on body weight, egg weight, or laying rate but feed consumption decreased (10% compared to the control; Ginzberg et al., 2000), likely because *Porphyridium* sp. is composed of 70% polysaccharides and can act as a fiber-rich diet for hens (Dvir et al., 2000). Despite no difference in feed intake, supplementing laying hens with 0.5% of the brown seaweed Undaria *pinnatifida* induced a higher laying rate (73.8% vs 65.2%) and egg weight (65.4 g vs 64.1 g) compared to the control group, with no effect on egg quality (eggshell color, strength, thickness, egg yolk color) (Choi et al., 2018). The authors hypothesized that this positive effect could be linked to the high oligosaccharide content in this brown seaweed (Heo et al., 2005). Finally, Carrillo et al. (2012) tested dietary supplementation with *Sargassum* spp. at 0%, 2%, 4%, and 8% in laying hens. Unfortunately, it resulted in a reduced laying rate, inversely proportional to *Sargassum* spp. incorporation rate (85.9% vs 91.3% for 8% supplementation).

Egg quality

Several studies have shown that algae can affect the quality of eggs. For instance, supplementing ad libitum-fed laying hens with 10% *Enteromorpha* sp. for 8 weeks induced a lighter yolk color compared to the control group (Carrillo et al., 2008). By contrast, 4% and 8% supplementation with *Sargassum* spp. in 35- to 43-week-old White Leghorn hens resulted in an improved yolk color (more intense) (Carrillo et al., 2012). These diverging results could be a consequence of the different types of carotenoids contained in these two seaweeds, *Enteromorpha* sp. containing mainly lutein, neoxanthin, and cryptoxanthin, which are particularly sensitive to sunlight and dried storage compared to the carotenoids contained in *Sargassum* spp. (mainly lutein, zeaxanthin, and fucoxanthin). Other studies have shown a positive effect of algae on yolk color, it becoming more yellow. This is the case for supplementation with *Spirulina platensis* (at 1.5%, 2%, and 3%) in 63- to 67-week-old Hy-Line W36 hens (Zahroojian and Morajev, 2013), with *Chlorella vulgaris* in 80-week-old Hy-Line Brown layers (Zheng et al., 2011), and with *Porphyridium* sp. (at 5% and 10%) in 30-week-old White Leghorn hens (Ginzberg et al., 2000). In addition to the effect on color, supplementing 42-week-old Highland Brown hens with 3% of the green alga *Enteromorpha prolifera* increased eggshell thickness and calcium content compared to the control group (Wang et al., 2013). Finally, Englmaierová et al. (2013) showed that 1.25% *C. vulgaris* supplementation in 25- to 39-week-old ISA Brown hens induced an increase in egg weight (62.3 g vs 61.1 g for control hens), shell weight (6.1 g vs 5.9 g for control hens), and yolk color, which was more intense for the supplemented group due to increased redness and yellowness.

Egg composition

Some algae are rich in fatty acids that can influence egg lipid composition. Several studies have reported that alga supplementation can significantly reduce egg cholesterol. This reduction varies between 19% and 23% depending on the study, the alga source, and the dietary supplementation level. Algae tested were *Spirulina platensis* (0.3%;

Sujatha and Narahari, 2011), *Sargassum* sp. (8%; Carrillo et al., 2012), *Enteromorpha prolifera* (3%; Wang et al., 2013), and *Sargassum dentifebium* (6%; Al-Harthi and El-Deek, 2012). The effect of algae on cholesterol in eggs is likely due to the effect of sterols, fatty acids, and polysaccharides on cholesterol synthesis in the liver of hens, either by direct interference with cholesterol pathways in the liver or by competition between diet and alga cholesterol in the small intestine absorption sites (Nishide et al., 2003). A 0.5% supplementation of the brown seaweed *Undaria pinnatifida* (Choi et al., 2018) induced higher albumin in blood as also observed in the case of dried brown seaweed (*Sargassum* spp.) supplementation (Rizk, 2017). Because albumin serves as a steroid and fatty acid transporter in the blood, increasing its amount with brown seaweeds could help laying hens to control their blood cholesterol. Alga supplementation also induces significant changes in the fatty acid composition of eggs. In the different studies reported by Fraeye et al. (2012), the n-3 LC FA content was 3.8- to 7.0-fold higher in groups fed with microalgae at various incorporation rates compared to the control group. Thereby, supplementation (2.9%) with *Schizochytrium* sp. in 21-week-old Line 477 hens affected both yolk color (redder) and n-3 fatty acid content. It increased the n-3 fatty acid content by a factor of 2.9 compared to controls, particularly long-chain fatty acids. However, such supplementation negatively affected egg taste, by inducing a fishy flavor (Baéza et al., 2015b). On the other hand, studies testing incorporation rates of between 2% and 4.8% microalgae in diets found no effect on the sensorial quality of eggs (Fraeye et al., 2012). Also, supplementing White Leghorn hens with 8% of the red alga Porphyridium sp. increased the linoleic and arachidonic acid content in eggs by 29% and 24%, respectively (Ginzberg et al., 2000). Similarly, 10% *Macrocystis pyrifera* supplementation in 35- to 43-week-old White Leghorn hens resulted in both increased lipid and n-3 fatty acid content (Carrillo et al., 2008), whereas 6% *Sargassum dentifebium* supplementation of 23- to 42-week-old Hy-Line hens decreased both triglycerides and n 6 fatty acid percentages of egg yolk (Al-Harthi and El-Deek, 2012). Moreover, the high level of carotenoids in microalgae can limit the susceptibility of eggs to oxidation. Finally, and as already mentioned above, supplementation with algae can also alter or increase egg carotenoid content and therefore yolk color. Providing 1% or 1.25% *Chlorella vulgaris* (respectively in 56- to 63-week-old and 25- to 39-week-old ISA Brown laying hens) significantly increased lutein, zeaxanthin, and beta-carotene in egg yolk (Englmaierová et al., 2013; Kotrbáček et al., 2015).

Hens' health

Algae are also considered as a potential source of prebiotic material. Therefore, they could be used to improve gastrointestinal health and the ability to absorb minerals and nutrients (Sako et al., 1999), to limit the overuse of antibiotics, and to reduce their adverse effects on animal microbiota and the risk of antibiotic resistance. Both *Chondrus crispus* and *Sarcodiotheca gaudichaudii* provided to 67-week-old Lohmann Brown classic hens at a 2% rate induced significant changes in the gastrointestinal tract. They increased villus height (+ 31%), cecum weight (+ 75%), and concentration of the beneficial probiotic intestinal bacteria *Bifidobacterium longum*, *Lactobacillus acidophilus*, and *Streptococcus* *salivarius* (Kulshreshtha et al., 2014). They also increased the short-chain fatty acid concentration in the gut microbiome that could help to limit colon diseases and preserve the integrity of the intestinal mucosa in chickens. The positive impact of algae on gut health and microbiota also induces decreased inflammation caused by pathogens compared to non-supplemented diets (Baurhoo et al., 2007). The microbial composition of 23-week-old Lohmann Brown laying hens' crop and cecum fed 5% *Chlorella vulgaris* was determined using 16S rRNA sequencing technology and compared to that of non-supplemented hens (Janczyk et al., 2009). The microalga *C. vulgaris* strongly affected cecum and crop bacterial population, especially lactobacilli species. This study also showed that hens' productivity and health, feed digestibility, and nutrient availability were directly correlated to changes in microbial community in the crop and cecum induced by alga consumption. Specifically, increasing the diversity of the universal bacterial community in the intestinal tract resulted in improved animal welfare and productivity. Supplementing 42-week-old Highland Brown hens with 3% *Enteromorpha prolifera* also increased the intestinal concentration of beneficial *Lactobacillus bacterium* and reduced the number of *Escherichia coli* in the feces compared to the control group not supplemented with algae (Wang et al., 2013).

In conclusion, algae seem to be a promising feed complement to improve egg quality and egg productivity of layers. Even though there is already evidence that algae can be beneficial for the digestive health of laying hens, it will be also important to evaluate their potential effect as a source of calcium. Indeed, seaweeds show a high content of calcium that is essential for laying hen production because it sustains bone health and eggshell quality, particularly in the current context which promotes the lengthening of laying hens' career.

Utilization for broiler chickens

Seaweeds have been used as feed additives for ruminants and pigs for a long time. Many benefits have been found for livestock species, providing protein and energy, and improving general and digestive health. An increasing number of studies have aimed to evaluate if seaweeds can be efficiently used to improve meat quality traits and animal immune status in poultry.

Growth performance

Whereas the use of microalgae as a new source of protein in poultry feed developed in the early 1980s (Lipstein et al., 1980; Becker, 1986), that of macroalgae (seaweeds) is more recent. Ventura et al. (1994) tested the possibility of complementing broiler and breeder diet with the green seaweed *Ulva rigida*. They concluded that incorporating this alga at 10% or more was not suitable because it significantly decreased feed intake and growth rate in both adult cockerels and 3-week-old broilers. This pointed out that alga incorporation rate is a crucial parameter to consider getting the most benefit for both the animal and product. A 4% supplementation of the green seaweed *Enteromorpha prolifera* increased feed intake and body weight gain in 35-day-old Arbor Acre broilers. It also enhanced nutrient availability and apparent metabolizable energy (Wang et al., 2013). In another study, Baéza et al. (2015a) reported that 2% supplementation with *Schizochytrium* sp. in Ross 308 broiler chickens improved their feed conversion ratio in the total rearing period thanks to decreased consumption for an equivalent growth rate. Yan and Kim (2013) did not observe any effect of dietary use of this microalga at 10 and 20 g/kg on the growth performance of Ross 708 chickens. In contrast, Ribeiro et al. (2013) demonstrated that the dietary supply of this microalga at 74 g/kg between 21 and 35 days of age had a positive effect on growth (+ 22%) and improved the gain to feed ratio (+ 15%) in comparison with control chickens fed with a diet containing 56.5 g/kg soybean oil. This beneficial effect of microalgae on the growth performance of chickens was confirmed in the study reported by Ribeiro et al. (2014).

In the same way, supplementation with 1% of the brown alga *Ascophyllum nodosum* increased the body weight of 42-day-old Ross broilers by 15% (Evans and Critchley, 2014). Such a positive effect on body weight was also reported with 1% supplementation of *Spirulina platensis* in 35-day-old Cobb 500 broilers (Ross et al., 1994). More precisely, increasing the rate of *S. platensis* supplementation (0.2%, 0.4%, and 0.8%) in growing Cobb 500 broilers (7 to 28 days) induced a proportional increase in body weight and decrease in food conversion ratio (**FCR**) (Jamil et al., 2015). At 28 days, body weight was 10% higher and FCR 10% lower (1.48 vs 1.65 for the control group) when the incorporation rate was 0.8%. Incorporation of 0.14% of the microalga *Chlorella vulgaris* in the diet of 42-day-old Ross 308 broilers also led to a reduction in FCR from 1.60 to 1.54 (Rezvani et al., 2012). When supplemented at a rate of 1%, *C. vulgaris* induced a 4.6% body weight increase in 28-day-old Ross broilers (Kang et al., 2013).

Meat production and quality

Adding algae to broiler diets can alter carcass and meat quality by changing their proximate composition or color. Thereby, supplementation with 4% of *Enteromorpha prolifera* in 35-day-old broilers decreased the abdominal and breast (pectoralis major) muscle fat content (Wang et al., 2013). Incorporating 3% of *Ulva lactuca* to replace 3% of corn in broiler diet from days 12 to 33 had no effect on feed intake and body weight but increased dressing percentage and breast meat yield and reduced the proportion of abdominal fat (Abudabos et al., 2013). The greater breast to fat ratio observed in the treated group could be a consequence of the hypocholesterolemic role of this seaweed due to its high soluble fiber and polyunsaturated fatty acid content (Carvalho et al., 2009). A 5% supplementation of *Spirulina platensis* changed the fatty acid composition of thigh muscles in 42-day-old broiler chickens by increasing arachidonic, docosahexaenoic, and total polyunsaturated fatty acids, and decreasing arachidic, eicosenoic, and total monounsaturated fatty acids (Bonos et al., 2016). Supplementing Ross 308 chickens with 2% *Schizochytrium* sp. increased the breast meat n-3 fatty acid content by almost four times compared to controls, mostly thanks to the linoleic and long-chain n-3 fatty acid richness of this microalga (Baéza et al., 2015a). Such supplementation also increased the ultimate pH of breast meat and decreased its lightness. It also strongly increased its oxidation susceptibility, with a TBARS (thio-barbituric acid reactive substances) value increased almost four times, confirming the observations reported by Ribeiro et al. (2013, 2014), but without impact on the sensory characteristics of breast meat. Only thigh meat from broilers supplemented with microalgae had an abnormal flavor after cooking (Baéza et al., 2015a). Due to their richness in carotenoids, some algae are likely to affect poultry meat color. This is the case for *S. platensis*, rich in zeaxanthin, that increased both the yellowness and redness of breast (pectoralis major) and thigh (sartorius) muscles of growing 21-day-old Cobb 500 broilers when supplemented at 0.4% (Toyomizu et al., 2001).

Broiler health

Chlorella vulgaris has been used to improve broiler health (Kang et al., 2013). Its incorporation at a rate of 1% in the diet of 28-day-old Ross broilers stimulated some innate immunity traits, such as white blood cells, lymphocytes, and plasma immunoglobulins IgA, IgM, and IgG that were respectively increased by 7%, 6%, and 25.5%. Similarly, supplementing 35-day-old Ross broilers with 0.5% *C. vulgaris* increased their IgG and IgM blood content by 46% and 107%, respectively (An et al., 2016). As leg weakness is a wide problem in poultry farming, algae could be an interesting alternative source of calcium. Nowadays, dietary calcium is mainly provided by limestone that decreases phosphorus digestibility, resulting in skeletal frailty. Red calcified seaweeds constitute an alternative organic and more digestive source of calcium for broilers that can contribute to a decrease in the use of mineral calcium and consequently improved phosphorus digestibility, resulting in better bone health and leg strength, and reduced lameness (Bradbury et al., 2012).

Beyond its effects on animal basal health status, alga supplementation can help fight negative effects due to heat or bacterial challenges. Supplementing heat-challenged Cobb 500 chickens (36 °C, 6 h/day between 38 and 44 days of age) with 2% *Spirulina platensis* increased the activity of both superoxide dismutase and glutathione peroxidase, resulting in improved antioxidant defense against free radical damage due to heat challenge (Mirzaie et al., 2018). The performance and health status of Arbor Acres broilers, inoculated with *Salmonella enteritidis* between 12 and 42 days, were also improved by supplementation with 0.2% of brown seaweed extract, rich in oligosaccharides (Yan et al., 2011). It muted the negative effects of *Salmonella* infection by upregulating mRNA expression of interferon-ɣ and interleukin-10 in the cecum and increasing blood IgA production. This resulted in decreased *Salmonella* colonization and improvement of intestinal barrier efficiency. It was also shown that the brown alga *Undaria pinnatifida* (provided at 2%) muted the effects of acute phase response following *Salmonella typhimurium* infection in Ross broilers (Koh et al., 2005). The acute phase response is an early defense system activated by infection and involving an innate immune response to prevent infection expansion and start the healing process (Cray et al., 2009). After 7 days, chicks fed or not with *U. pinnatifida* were infected with *Salmonella* and their acute phase response was activated, leading to changes in plasma protein concentration. Brown alga supplementation did not affect chicken performance but increased protein retention by decreasing protein breakdown and lowering nitrogen balance.

For broiler chicken production, algae are likely to be valuable complements in order to improve growth, feed conversion ratio, meat quality, and basal health. However, when included at too a high concentration in the diet, algae can have a deleterious effect on feed consumption, body weight gain, and meat quality due to their composition and digestibility.

Conclusion

The aim of this review was to present the current knowledge regarding the effect of using macro- and microalgae in broiler and laying hen nutrition. The literature is quite abundant and generally shows a beneficial effect of supplementation on many parameters related to animal performance, basal health, and product quality. This review does not particularly reveal the functional properties that are linked to the color of algae: green, red, or brown. Many varieties of algae exist across the world, whose chemical composition and digestibility are very different and likely to be influenced by how they are obtained. For this reason, acquiring knowledge on their composition is very important to evaluate their potential benefit for poultry and more generally animal nutrition. According to published results, it seems that a maximum incorporation rate of 2% for microalgae or a range between 1% and 5% for macroalgae is suitable for both laying hens and broiler chickens, even though these ranges greatly depend on the type of algae used and the expected benefits for poultry production.

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Reproductive biotechnologies

Molecular and cellular aspects of avian sex determination (M. McGrew)

Mike MCGREW

Tuanjun Hu, Maeve Ballantyne, Michael Clinton, Mike McGrew

The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, 5 Easter Bush Campus, United Kingdom
mike.mcgrew@roslin.ed.ac.uk

Summary

It has been proposed that both somatic and germ cells have a sex identity in avian species. Researchers have investigated the role of chromosomal sex on avian germ cell differentiation in opposite sex hosts. They have found that male (ZZ) germ cells will form functional oocytes in a reverse sex host but female (ZW) germ cells do not form spermatozoa. We recently developed genetically sterile layer roosters and hens as surrogate hosts for primordial germ cell (PGC) transplantation. Using in vitro propagated PGCs, we found that female sterile hosts carrying chromosomally male (ZZ) PGCs formed functional oocytes and eggs, which gave rise to 100% male offspring after fertilisation. We also observed that chromosomally female (ZW) PGCs carried by male sterile hosts formed functional spermatozoa and produced viable offspring. Both results support previous findings. The Z chromosome gene DMRT1 is important for somatic cell differentiation of the gonad. A shRNA knockdown of DMRT1 expression led to masculinisation of the embryonic gonads. We knocked out DMRT1 using CRISPR/Cas9 and found that males containing a single copy of the DMRT1 gene formed both ovaries and oviducts but overall the body had male sexual characteristics. These results demonstrate that avian somatic cells have a sexual identity but avian germ cells do not.

Key words: chicken, DMRT1, sex determination, gametogenesis

Chicken is the major poultry species generating both meat and eggs for consumers. Chickens number over 60 billion and accounts for 34% of global meat production (Pawar et al 2016; Richie and Roser, 2021). Thus, fertility for the formation of fertile eggs and functional spermatozoa is important for effective poultry production. The reproductive cells of the bird, the germ cells, contain the genetic information that is transferred from one generation to the next. The differentiated germ cells, the highly specialised sperm and egg, each carry a haploid genome and recombine to form the diploid fertilised bird egg. Cryopreservation of adult germ cells, sperm and oocytes, is now routine in ruminant livestock. However, in avian species, the cryopreservation of the mature gametes is inefficient. It is currently impossible to freeze the large yolk-filled bird egg. The cryopreservation of chicken semen is also inefficient for some chicken breeds (Thelie, Bailliard et al. 2019). This inefficiency may be due to both the length of the chicken oviduct

(20-40 cm), the prolonged storage of semen in specialised oviductal glands, the disruptive effects of the freezing and thawing process, and the contraceptive effects of cryoprotectants (e.g. glycerol) in the freezing media. (Blesbois, Grasseau et al. 2008, Matsuzaki, Hirohashi et al. 2021).

Sexual determination of the germ cells

During embryogenesis the germ cells become restricted to a male or female fate after completing their migration to the gonadal anlagen (Adams and McLaren, 2002). Post-migratory germ cells begin to express ovarian or testes specific genes appropriate to their gonadal environment, which culminates in embryonic entry into meiosis for female ovarian germ cells and meiotic quiescence for male testicular germ cells until the onset of sexual maturation. The onset of sexual breeding is accompanied by oocyte maturation in females and spermatogenesis in males.

Are germ cells determined to form male or female gametes depending on their sex chromosomes? In some teleost fish species with genetic sex determination, germ cells from adult gonads will form functional oocytes or spermatozoa in the reverse sex gonads (Okutsu et al., 2006; Dranow et al., 2013). In amphibian and reptilian species, the findings are species specific; in many amphibian and reptilian species without genetic sex determination, environmental factors can determine the development of a sexual phenotype and gamete formation. In amphibian and reptilian species with genetic sex determination, sex-reversed animals are fertile in many species, suggesting that in most amphibian and reptilian species germ cells are bipotential in fate and can form either sperm or eggs independent of sex chromosome composition (Wallace et al., 1999; Flament, 2016). In mammalian species, however, PGCs are sex-restricted in the generation of functional gametes. Germ cell transplantation experiments in mice have shown that chromosomally male (XY) germ cells formed ovarian follicles but these have seldom been observed to produce mature oocytes or offspring (Ford et al., 1975; Eicher et al., 1982). Similarly, chromosomally female (XX) germ cells colonized the male gonads and adult testes, but could not complete meiosis which can be attributed to either the lack of Y chromosome gene products or the presence of two copies of the X chromosome (Palmer and Burgoyne, 1991). These results suggest that sex-restricted gamete differentiation was acquired in mammals during sex chromosome differentiation and vertebrate speciation.

In bird species, males are the homogametic sex and contain two Z sex chromosomes while females are heterogametic and contain one Z and one W chromosome. Avian PGCs form precociously in the pre-laid egg before the formation of the embryo. In chicken, migratory PGCs at migratory have a sex-specific proteome (Soler et al., 2021) suggesting that they also have a sex identity. Supporting this hypothesis, germline transmission of in vitro propagated donor chicken PGCs was not observed in wildtype or irradiated reverse sex hosts (van de Lavoir et al., 2006; Macdonald et al., 2010). Similarly, genetically sterile DDX4 knockout female surrogate host chicken carrying in vitro propagated donor male PGCs did not contain mature oocytes in their ovaries and did not lay eggs (Woodcock et al., 2019). In contrast to these findings, female donor chicken PGCs transplanted into wildtype male surrogate host produced semen PCR positive for the W chromosome (Tagami et al., 1997). However, few W-bearing differentiated spermatozoa were detected. Migratory PGCs directly transplanted into partially sterilized opposite sex surrogate host embryos also formed functional gametes and offspring in both male and female reverse sex hosts albeit at extremely low frequencies (Naito et al., 1999). In a similar result, we demonstrated that in vitro propagated male layer PGCs formed oocytes and hatched chicks when transplanted into irradiated female layer surrogate hosts (Liu, Chang et al., 2017). In our recent work, (Ballantyne et al (2021)) we found that both male and female PGCs injected into opposite sex hosts form functional gametes. This result depended on the surrogate host completely lacking endogenous germ cells.

Sexual determination of avian somatic cells

Primary sex determination refers to the generation of testes in males and ovaries in females. While the factors that regulate this developmental process are generally conserved in vertebrates, the mechanisms that initiate the process can vary considerably between species. In mammals, gonadogenesis is considered a linear process dependent on a ‘switch’ mechanism based on the Y-chromosome Sry gene. In the XY embryo, Sry upregulates the transcription factor Sox9, which induces Sertoli cell fate, anti-Mullerian hormone (AMH) expression, and testis differentiation. In the XX embryo (lacking Sry), FoxL2 signalling is a major promoter of ovarian differentiation. In mammals, the male and female sexual phenotype is dependent on the nature of the gonad formed: testis in male and ovary in female. Hormones secreted by the gonads promote development of reproductive structure and also determine the overall body sexual phenotype (reviewed in Sekido and Lovell-Badge, 2013). The extent of hormonal control of secondary sexual characteristics and seasonal control of mating behaviours is highly divergent in animal species. Analysis of half-male: half-female natural gynandromorph chickens has demonstrated that avian somatic cells acquire a cell autonomous sex identity (CASI) at fertilisation and suggest that the development of avian sex characteristics are independent of gonadal sex (Zhao et al., 2010).

It is widely recognized that such primary sex determining mechanisms evolve rapidly, and this is exemplified by marked differences between mammals and other vertebrates (Bachtrog, D., et al., 2014). For example, concerted efforts to identify Sry-like master switch genes in other species have been largely unsuccessful. In birds, no switch gene, analogous to the mammalian Sry, has been identified on the Z chromosome and no female master regulatory gene has been found on the gene-poor W chromosome.

Evidence of how sex-determining systems have evolved may also be deduced from some recent surprising observations relating to mature gonads. It was previously considered that gonadal fate in mammals, once established, was permanent, however, a series of studies in adult mice have revealed that gonadal sex is plastic and must be actively maintained throughout adult life (Lindeman et al., 2015; Matson et al., 2011). The available evidence suggests that testicular and ovarian fate in mice is maintained by expression of Dmrt1 in Sertoli cells and Foxl2 in granulosa cells, respectively. Ablation of Dmrt1 induces Sertoli to granulosa cell transdifferentiation, while ablation of Foxl2 results in granulosa to Sertoli cell transdifferentiation. Conditional inactivation of Foxl2 in postnatal ovaries has shown that Foxl2 maintains granulosa cell identity by cooperating with Estrogen Receptor beta (ERβ) in repressing transcription of Sox9, and studies of Dmrt1 null mutations have demonstrated that Dmrt1 maintains male cell fate by activating transcription of male-promoting genes (e.g. Sox9, AR) and by repressing transcription of female promoting genes (e.g. Foxl2, ESR1, ESR2, Wnt4, Rspo1). Moreover, transdifferentiation of Sertoli cells to granulosa cells in the Dmrt1 null mice can be suppressed by ablation of Foxl2, while over-expression of Dmrt1 in female granulosa cells is sufficient to cause transdifferentiation to Sertoli cells and to induce transcriptomic changes similar to those seen with the Foxl2 deletion, suggesting Foxl2 as a major ovarian determinant. It appears that gonadal sex in the adult mammal, is dependent on the balance between Dmrt1 and Foxl2 regulating the expression of male-promoting and female-promoting genes. Much of our understanding of sex determination, sex differentiation and gonadal sex maintenance, comes from studies on the mouse, but questions have been raised as to the universality of this model system. Although Dmrt1 and Foxl2 are dispensable for the embryonic phases of sex differentiation in the mouse (Raymond et al., 2000; Schmidt et al., 2004), these factors have been shown to play key roles in embryonic gonadogenesis in several vertebrate species and even a few mammals. For example, Foxl2 is a primary ovarian determinant in the goat (Boulanger et al., 2014) and human XY patients hemizigous for Dmrt1 are born as sex reversed females, suggesting a potential embryonic role for Dmrt1 (Tannour Louet et al., 2010). The embryonic ovary in many mammals (including human and goat) and non-mammalian vertebrates produces oestrogen, but this is not the case in the mouse (Greco et al., 1994) and, consequently, murine ‘knockouts’ of p450 aromatase and estrogen receptors only show a phenotype after birth. Moreover, in some mammals and in lower vertebrates, Foxl2 has been shown to activate expression of p450 aromatase, the enzyme required for estrogen production (Fleming et al., 2010; Oshima et al., 2008; Wang et al., 2007). Indeed, the requirement for DMRT1 and FOXL2 in adult mammalian gonads may represent an evolutionary residue of major roles in primary sex determining mechanisms in lower vertebrates, where a plasticity is often evident during embryonic stages (Heule et al., 2014). It is possible that the insensitivity to estrogen signalling that has developed in mouse primary sex determination reflects the evolution of a different hierarchy of control elements in the initial stages of gonadogenesis, and made the Dmrt1/Foxl2 antagonism dispensable to primary events in mouse sex determination. One hypothesis proposes that primary gonadal sex determination depends on the dosage of one or more genes on the Z chromosome, with males (ZZ) having a higher dose than females (ZW) (Guioli et al., 2014). Dmrt1 is the best candidate for a dosage dependent mechanism: it is Z-linked, expression is restricted to gonads and Mullerian ducts, and it is expressed at higher levels in the male than in the female at the time of sex determination (Smith et al., 1999). A series of studies were performed where Dmrt1 levels were manipulated by electroporating or transducing constructs into developing embryos in ovo. These showed that, in the embryo, reducing levels of Dmrt1 leads to a degree of feminisation of the ZZ male gonad and that overexpression leads to masculinisation of the ZW gonad (Lambeth et al., 2014). While these data support a Z-dosage based mechanism, they do not constitute proof that Dmrt1 is the sex-determining gene in chick. Most notably, it has yet to be demonstrated that a threshold of Dmrt1 expression is the key regulator of sex determination. We have recently demonstrated that dosage of DMRT1 controls sex determination in chicken (Ioannidis et al, 2021). We used a CRISPR-Cas9 gene editing approach to both reduce and deplete functional chromosomal copies of DMRT1 in males and females, respectively. We mutated one allele of DMRT1 in male PGCs as DMRT1 is important for male gametogenesis. The targeted PGCs were injected into genetically engineered sterile surrogate male embryos. These embryos were hatched and raised to sexual maturity then crossed to wild type females to generate the following DMRT1 genotypes: ZZ-DMRT1+/+, ZW DMRT1+/- (wild-type males and females), ZZ DMRT1 -/+ (Heterozygous [Het] male) and ZW DMRT1-/o (knockout [KO] female). The males with a single functional copy of DMRT1 showed complete primary sex reversal i.e. ovary development. The females lacking DMRT1 also showed ovary development, but with defective germ cell development. This analysis demonstrated that DMRT1 is a crucial factor in testis development. Importantly, the secondary sexual features of the gonadal sex-reversed birds were not affected, confirming our hypothesis that avian somatic cells have a sex identity.

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Reproductive physiology

Regulation mechanisms of sperm acrosome reaction in Chickens: the role of membrane rafts (A. Asano)

Atushi ASANO

Faculty of Life and Environmental Sciences
University of Tsukuba, Ibaraki 305-8572, Japan

Email: asano.atsushi.ft@u.tsukuba.ac.jp

Abbreviated title: Membrane regulation of sperm AR

Summary:

Transcriptionally and translationally inactive, sperm function relies on extra-cellular stimuli to activate signaling cascades preassembled into specific areas where need a function. Despite of the requirement of acrosome reaction (AR) for sperm prior to penetration into ovum, the mechanisms for how signaling pathways are coordinated to results in the exocytotic event is not fully understood. Membrane rafts are functional membrane microdomains, and they play a role in regulation of diverse cellular processes. Recent our studies have showed the involvement of membrane rafts in AR via regulating several signaling cascades in chicken sperm. The presentation will describe membrane lipid regulation of AR induction and its application to sperm preservation technology in chickens.

Keywords:

Acrosome reaction, chicken sperm, membrane rafts, signal transduction, sterol

Text:

Once ejaculated from the male reproductive tract, avian sperm undergo an acrosome reaction (AR) by interacting with the inner perivitelline layer (IPVL), which enables them to release proteolytic enzymes that hydrolyze the inner perivitelline layer (IPVL) and then penetrate into oocyte. Several studies concerning avian sperm AR led to view the involvement of many signaling cascades with both similarity and distinction from known mechanisms underlying mammalian AR (Asano and Tajima 2017, Lemoine, et al. 2009). However, it is not fully understood how the signaling cascades are coordinated to result in AR in chicken sperm that ensure fertilization potential for prolonged period.

Transcriptionally and translationally inactive, sperm function relies on external stimuli to activate preassembled cellular pathways into specific compartments. How is the transmembrane signaling propagated? Membrane rafts are specific membrane microdomains enriched in sterols, ganglioside G_{M1} and functional proteins, and play important roles in regulation of diverse cellular processes. Accumulated studies attempted to determine the roles of sperm membrane rafts in mammals have demonstrated the functional involvement in the multistage of fertilization (Asano, et al.

2013, Cohen, et al. 2014, Khalil, et al. 2006, Thaler, et al. 2006). This led us to investigate the involvement of membrane rafts in signaling cascades preceding with AR in chicken sperm. Our biochemical, proteomic and functional analyses have shown membrane rafts-mediated regulation of the cellular cascades resulting in elevation of acrosomal responsiveness (Asano, et al. 2016, Priyadarshana, et al. 2020, Priyadarshana, et al. 2018, Ushiyama, et al. 2019, Ushiyama, et al. 2017a, b).

Because of the functional importance of cellular membrane, it is known to be a primary site that occurs cryodamage in sperm. However, the mechanism for how it is initially induced is poorly understood. Recent our findings, in conjunction with results from mammals, have shown a potential of improved post-thaw survival and function in chicken sperm by modulating the nature of membrane microdomains (Moraes, et al. 2010, Ushiyama, et al. 2016, Ushiyama, et al. 2017b).

The presentation will describe the membrane regulation of AR and its application to sperm preservation technology in chickens.

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Meat quality - slaughter and processing

Trends and perspectives for poultry processed products (S. Barbut)

Shai BARBUT

Food Science Department
University of Guelph
Ontario, Canada
sbarbut@uoguelph.ca

Major changes in poultry meat production have been seen over the past few decades. Between 1950 and today, the average broiler weight increased from 1.5 kg to 3.0 kg, while market age decreased from 75 to about 40 days. Also, in broiler primary processing plants line speed has more than quadrupled over the same period, while more cutting up and further processing have been introduced. For example, in 1960 85% of the broilers were sold as whole birds, and today they only represent < 10% of the total North American market. Big shifts have also been seen in marketing chicken wings (used to be a wasted product and today are sold as a premium product), dark meat which is now becoming more expensive than white meat in N. America, and the price of breast fillet coming down since 2015 from $5 to $1 and now back to $2 / lb. These changes are the result of improvements in breeding, nutrition, processing, etc., and have major impacts on the industry in terms of becoming more competitive, lowering production cost and improving efficiency.

Computers and robots can now be seen in hatcheries, barns, and in processing plants where full automation of certain areas (plucking, evisceration, cut up) is becoming the norm. The recent pandemic has increased the rate of implementing automation in processing plants, as labor availability and demands to keep works further apart are big challenges in the poultry and red meat industries. A limiting factor in this acceleration is that many companies are now waiting for equipment to arrive as machinery supply is now facing challenges due to shortages of components and problems in the international supply chain. With consumers, we see a continuous demand for more convenience type products that require minimum or no preparation, which has also opened a big market for the further processed poultry meat segment. Today fully cooked nuggets, sausages and cold cuts are very popular, while providing higher margins to processors. Investments in automated lines to produce such products (e.g., 0.2 M nuggets pre/hr without any person touching the product; fully automated co-extrusion sausage lines working 24/7), as well as developing special ingredients and flavors are now a big driver force in the industry. Implementing more of the Industry 4.0 concepts in primary and secondary poultry processing is evident around the world (e.g., diagnostics and problem solving of a meat plant in one continent are done instantaneously in another continent via the internet).

In comparison to other meats, poultry production keeps on rising and has now surpassed beef all around the world, and pork in many countries. This growth has not been without challenges. One of the largest today is the appearance of myopathies such as the so-called 'white striping' and 'woody breast' fillets of chickens and turkeys. The magnitude of these challenges, new ways to sort out the meat (e.g., NIR spectroscopy), and current solutions for dealing with such meats at the meat processing plant will be discussed. In addition, potential ideas for solving these challenges in the future (e.g., use of genetics, nutrition, husbandry), and the move towards more slow-growing breeds in some countries, will be presented.

Another challenge for the industry is the explosion in the number of the Stock Keeping Unit (SKU) which in some large broiler processing companies can reach 500 (e.g., whole / half / quarter chicken, chicken breast with / without bone, with / without skin). This requires a much better understanding of the raw materials quality coming into the plant and presents new challenges to breeding companies, farmers, processors and distribution channels. Overall, it is amazing to think that 75 years ago products such as chicken hotdogs / frankfurters were unknown to the public and today they capture over one quarter of the market.

In summary, the global poultry industry has experienced tremendous growth, and managed to overcome many challenges in the past, so there is no reason to believe it will not continue to thrive.

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Genetics: new traits

Genetics of new measurements: examples of implementing precision livestock farming tools in poultry breeding (T. Van De Braak)

Teun VAN DE BRAAK

T.G.H. van de Braak, Institut de Sélection Animale B.V. a Hendrix Genetics company, Boxmeer, the Netherlands – EU

Corresponding author: teun.van.de.braak@hendrix-genetics.com

Abbreviated Title: Genetics new phenotypes poultry

Summary

In recent years, many high-tech innovations have been introduced in animal husbandry. From in depth image capturing and analyses to robotics, and from big data to smart sensors. These new technologies allow to capture phenotypes that have not been recorded before. Resulting in the investigation of the heritability of these new traits for breeding companies and next to the heritabilities the genetic correlations with existing traits. Implementing new technologies isn't always a smooth process. The complexity of a farm environment (think of higher levels of small particle emissions impacting the technologies when compared to high-tech and extremely clean laboratories), the difference in scale (comparing breeding facilities with thousands of individuals versus research set-ups at universities) to identify individuals and finally the unpredictability of the animal itself in its behavior can make it challenging to quickly adopt the latest innovations in poultry breeding. In this talk several examples of novel ways to capture high quality phenotypes will be provided.

Key words: Poultry, breeding, genetic selection, precision livestock, phenotypes

Text

Accurate data collection is at the heart of all our breeding programs. With the right data, the geneticists can select the best birds with improved animal welfare, higher productivity, and lower carbon footprints compared to previous generations. The implementation of blockchain, artificial intelligence, robotics, vision technologies, big data and the use of sensors allow breeding companies to meet the need to stay connected to the latest development in the tech world. The aim of breeding companies is to examine these new technologies and try to understand how these concepts could enhance the field of animal genetics and poultry breeding. Open innovation and fostering collaboration with partners inside and outside the poultry sector allow animal breeders to implement techniques and technologies that are not perse within the traditional way of looking at birds and recording traits.

Machine vision is one of the technologies that has been adopted in recent years. Machine vision refers to the technologies and methods used to provide imaging-based automatic inspection and analysis. By adopting machine vision in the breeding program for laying hens, higher accurate data can be collected on exterior egg phenotypes. Automating the egg grading process has resulted in removing the possibility for human error and subjectivity. Each external egg quality evaluation can now be completed with a high amount of measurable consistency and repeatability, thereby improving the quality of data largely, and removing the human bias. It also allows to adopt new traits which were harder to score with just making use of the human eye before, like the gloss of the eggshell and the variation in eggshell color of each individual eggshell. As with other traits related to egg quality, heritabilities of the various traits that are captured via machine vision are moderate.

Another technique that got implemented in poultry breeding programs is MRI, which stands for Magnetic Resonance Imaging. The use of MRI allows to look under the surface of the eggshell, without breaking it. The MRI technology is far from new, as almost every hospital around the globe uses MRI technology for scanning organs and tissues. The potential is high, but the major disadvantage is the high investment cost. By making use of artificial intelligence affordable, fast, and versatile MRI's have been developed in recent years, allowing the tools to be implemented in hatcheries as well. By generating images for algorithms and deep learning, the current MRI tools allow for checking hatching eggs without breaking them. It allows to identify fertility prior to setting eggs, the viability of the germ disc and when performed at later stages the gender of the chick. This does not only benefit selection traits related to fertility and hatchability, but also has its direct benefits for hatchery optimization and utilization, as unfertile eggs do not need to be incubated.

Next to MRI, also Computed Tomography (CT) scanning technology got adopted in poultry breeding programs, especially in turkey and broiler breeders. Hybrid Turkeys has implemented CT technology to get in-depth information on the various aspects of a turkey's body such as skin, bones, body composition, airways, and organs. By scanning body composition, economic traits such as breast and thigh yield can be recorded without culling the bird. To analyze the yield information of individual birds scanned, Hybrid uses the 3D models created to estimate the total grams of breast meat per kilo of live weight. This information is used to analyze and compare birds and aid in the selection process. The use of CT technology comes as an enhancement, not a replacement to the current data collection efforts in the breeding program thus accelerating progress on these highly desirable yield-based traits. Although meat yield is a popular trait, much more information on attributes like skin, bones, airways, and organs can be collected via CT scanning. This additional data addresses key performance factors such as livability and overall health and welfare of a turkey. As an example, assessing heart size and shape and how it contributes to getting blood flow to the muscles can provide interesting insights to address livability. Or consider bone angulation and density and how this can indicate the ability of a bird to support increased yields. Improvements in livability help not only to improve total flock economics, but also have a positive impact to environmental sustainability in that resources utilized throughout the life of a turkey are not wasted. From the data available through CT scans, several new possibilities exist to improve performance, efficiency, and welfare traits. In the end it is about utilizing this technology to create a more sustainable turkey with enhanced economic, social, and environmental gains while still meeting the needs of the market.

IMAGEN, (AnIMAl Group SENsor) is a clear example of a multi-faceted, long-term study that combines animal breeding with advances in sensing and AI technologies to help in the transition towards sustainable livestock production. There is an urgent need to respond to societies' demands relating to animal welfare. To address this, the animal-protein sector continues to transition to "welfare-friendly systems", such as cage-free housing systems for laying hens and slower growing broilers (growth below 50 grams a day) in European countries. This change presents a challenge to understand and predict animal behavior in these relatively new environments. Advancement in vision, sensing, and AI (artificial intelligence) technologies offer a crucial opportunity to develop new methods to automatically detect and analyze animal behavior. Better understanding of social interactions between animals in social groups will result in reduced instances of harmful animal tendencies such as feather pecking in laying hens and tail biting in swine. To address these issues, Hendrix Genetics have joined the IMAGEN project. By combining sensing and AI technology with animal breeding and genetics, this project aims to improve the health and welfare of livestock and reduce the ecological footprint of our food production. Hendrix Genetics will be closely involved in one of the sub-projects focused on behavioral interactions in laying hens. The project started in 2021 and will span five years and contain tasks around developing sensing and AI technologies for automated behavior detection in laying hens, the behavioral research of the emergence and spread of behaviors in laying hens and the genetic modelling of the emergence and spread of behaviors in laying hens. The hope is that IMAGEN will result in a prototype hardware/software system for the automated detection of behaviors of laying hens kept in large groups. Building on the data generated by these technologies, we will develop mathematical models and data analysis methods to understand the behavior of groups of animals and identify the genetic and environmental factors determining the health, welfare, and ecological footprint.

Another example of improving the breeding of laying hens by using innovative tracking and data analyses strategies is the HenTrack project, headed by Dr. Michael Toscano, Group Leader of the Center for Proper Housing of Poultry and Rabbits (ZTHZ). The HenTrack project will utilize large-scale commercial facilities to conduct detailed, continuous observations of individual hens over the entire laying period. Previous research by Rufener and colleagues (2018) has shown highly consistent behavioral patterns of individual hens that nonetheless are very different between hens. Most critically, the patterns appear to be linked to basic biological processes, e.g., the epigenome (Guerrero-Bosagna et al., 2020), and associate with conditions relevant to animal welfare, like bone injury (Rufener et al., 2019). Data generated by HenTrack includes information on how animals acclimate to standard commercial stressors such as dietary changes and vaccinations, as well as variation in use of their environment, including access to outdoor areas. Information collected at the research facilities are combined with traditional breeding evaluations in small family groups.

Above are just few examples on how breeding companies are adopting "new" technologies in their breeding programs. It is evident that poultry breeding is more complex than ever before. Changing requirements that society puts on the way that poultry is kept requires sufficient focus and attention from breeding companies, to safeguard that the poultry are fit for the future. By adding new traits and technologies it remains evident that genetic variation is essential in poultry breeding, and that balanced breeding and selection is key to maintain viable populations that are fit for the future and can meet the needs of a rapidly changing society.

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