Combined workshop of WPSA

"Fundamental Physiology and Perinatal Development in Poultry"

And

"Incubation and Fertility Research Group"

28 – 30, August 2019

Tours, France

WPSA France

Book of Abstracts

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TOURS, FRANCE

Edited by

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for the French Branch of WPSA

WPSA France

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Foreword

Dear friends and colleagues,

The French branch of the WPSA and the "Incubation and Fertility Research Group" (#6, IFRG) and "Fundamental Physiology and Perinatal Development in Poultry" (#12, PDP) Working Groups of the European Federation of WPSA are very pleased to welcome you at the Univers hotel, Tours, for this new edition of the combined IFRG-PDP workshop.

This joint conference is held for the first time in France, and we are happy to warmly welcome you in the Loire Valley, heart of France, from the 28th to the 30th of August, 2019.

This workshop inherits a long tradition of meetings between scientists and the poultry production sector. It will allow to stay ahead of the latest scientific knowledge and to support fruitful networking activities too.

We kept the format used in the previous symposia. The program includes four invited keynote lectures presenting perspectives on current issues of hatchery practices and avian physiology, epigenetics, egg quality and embryo development, communications on current topics of interest concerning reproduction, incubation and physiology, posters with an observation session introduced by a short oral presentation for each poster. We want really to thank the participants and speakers for their engagement to make it a successful scientific symposium.

We would like to express our thanks to the European Federation of WPSA, the Foundation for Poultry Science and to our sponsors for their support and contribution for this event.

We are sure that you will enjoy both the scientific program, the social program and the beauty of the Touraine environment in the so-called "French Kings Valley".

You are very welcome in the Loire Valley for the combined IFRG-PDP meeting of WPSA

Anne Colin-Chenot
Chair of the Scientific Committee
IFRG-PDP 2019

Christophe Bostvironnois
President of the French WPSA Branch

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1st session:

Dynamics of hormones and nutrients in the egg, the embryo and the chicken

Regulation of the hepatic metabolic switch during perinatal development of the chicken

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The transition from embryonic to posthatch development in the chicken represents a massive metabolic switch from primarily lipolytic to primarily lipogenic metabolism. This metabolic switch is essential for the chick to successfully transition from the metabolism of stored egg yolk to the utilization of carbohydrate-based feed. However, regulation of this metabolic switch is not well understood. We used high-throughput RNA sequencing to characterize expression profiles of mRNA and microRNA (miRNA) in liver during late embryonic and early posthatch development of the chicken. We found that expression of over 800 mRNAs and 30 miRNAs was altered in the embryonic liver between embryonic day 18 (e18) and posthatch day 3 (d3), and many of these differentially expressed mRNAs and miRNAs are associated with metabolic processes. We confirmed the regulation of some of these mRNAs by miRNAs expressed in a reciprocal pattern using luciferase reporter assays. To further elucidate the roles of miRNAs in the metabolic switch, we used delayed feeding for 48 h to impede the hepatic metabolic switch. We found that hepatic expression of several miRNAs was affected by delaying feed consumption for 48 h. For example, we found that delayed feeding resulted in increased miR-20b expression and conversely reduced expression of its target FADS1, an enzyme involved in fatty acid synthesis. Interestingly, the expression of a miR-20b regulator FOXO3 was also higher in delayed fed chicks. To further characterize the regulation of the hepatic metabolic switch, hepatocytes were isolated from e18 and d3 chicks and treated in culture with insulin, glucagon, growth hormone, or corticosterone, and expression of metabolic genes was measured. We found that the metabolic switch between e18 and d3 was associated with changes in hepatocyte responsiveness to each hormone evaluated. Our studies demonstrate that many transcriptional, post-transcriptional, and endocrine mechanisms form a complex regulatory network that controls lipid and glucose metabolism during the embryonic to posthatch transition in chickens.

Keywords: Liver; Metabolism; Hatching; Genomics

Metabolomic analysis of nutrient sources in the embryonic egg of two divergent lines for meat ultimate pH

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Divergent selection on the ultimate pH (pHu) of the breast muscle has allowed the creation of the pHu+ and pHu- lines, which represent a unique model to study the genetic and physiological control of energy stores and meat quality in chicken. Indeed, pHu+ and pHu- chicks (presenting low and high-energy status, respectively) exhibit different nutrient and hormone response capacities at hatch.

The avian egg forms a natural chamber that contains all the elements that are necessary for the survival and development of the embryo. During the first two weeks of development, nutrients are mainly provided by the lipids and proteins contained in the yolk. In the last third of development, embryo will use other nutrients present in the egg white and amniotic fluid. We hypothesized that a variation in these nutrient sources could contribute to metabolic and developmental differences that are present at hatch between the pHu+ and pHu- lines. To address this question, we analyzed the physical and chemical characteristics of the yolk and performed some metabolomic analyses (1H-nuclear magnetic resonance, NMR) at E0 (the first day of incubation) and E10 (after 10 days of incubation) for yolk and at E10 for amniotic fluid.

Metabolomic analysis evidenced changes in yolk composition between E0 and E10 stages. However, no difference in metabolomic profile was found between the two lines. In contrast, chemical analysis revealed a higher lipid percentage at E0 in the pHu+ line (32,9%) that appeared particularly low in the pHu- line (27,7%). On the other hand, analysis by 1H-NMR spectroscopy of the E10 amniotic fluid showed a different metabolic signature between the lines with leucine, isoleucine, oxoisocaproate, citrate and β-glucose being superabundant in pHu+ line while choline and inosine being superabundant in pHu- line.

These results highlight quantitative and qualitative differences in the nutrients potentially available to developing embryos, which could explain metabolic and developmental differences between the pHu+ and pHu- lines. The molecular characterization of the different compartments of the egg will help in understanding the metabolic orientation of the embryos (according to their nutrient sources and genetics) and could contribute to identify biomarkers reflecting the animal's energy status in ovo.

Key words: Embryo; Development; Egg; Nutrient; Metabolome; Energy status

Intestinal brush border maturation during the peri-hatch period: the effects of in-ovo feeding of L-Glutamine

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The ability of chicken to absorb nutrients is governed by the functional surface area of small intestinal enterocytes, termed the intestinal brush border. This region is comprised of microvilli at enterocyte apical membranes, which amplify their surface area and contain nutrient transporters. The peri-hatch period is a critical phase in which the small intestine prepares for post-hatch nutrition. Therefore, inducing early brush border maturation during this period will improve digestion and provide additional support for chicks during the critical first week post-hatch. This may be accomplished by in-ovo feeding (IOF) of glutamine (Gln), since Gln has been found to induce enterocyte growth and promote microvilli integrity in mice models.

The aim of this study was to characterize brush border maturation during the peri-hatch period and examine the effects of IOF of Gln. The IOF procedure was conducted in Cobb500 eggs (n=120) at E17.5 in 3 groups: 1% glutamine in 0.4% NaCl (IOF-Gln), 0.75% NaCl (IOF-NaCl) and no IOF (control). Jejunum sections from all groups were sampled at peri-hatch ages and analyzed by light microscopy, scanning electron microscopy (SEM) and qPCR. Genes examined were brush border structural genes Villin (filament bundling protein) and Ezrin (filament stabilizing protein) and enterocyte functional gene PepT1 (oligopeptide transporter).

Results in the control group showed that at E17, enterocytes were lined with short microvilli, prior to villi formation. By DOH, villi emerged and microvilli increased in height and uniformity. At D3, microvilli heights increased by 51%; MAF, the factor by which microvilli amplify enterocyte surface areas, increased 1.8-fold; total apical surface area (enterocyte surface area X MAF) increased 4.7-fold. Expression of Villin, Ezrin and PepT1 increased significantly towards hatch ($P<0.05$). IOF-Gln significantly increased the brush border surface area at DOH, compared to the control and IOF-NaCl groups: villi heights and villi/crypt ratios increased by 20% and microvilli heights increased by 30% ($P<0.05$). Furthermore, MAF and total apical surface area were 1.2 and 1.6-fold higher, respectively. Expression of Ezrin and PepT1 in the IOF groups was significantly higher compared to the control group ($P<0.05$).

In conclusion, IOF-Gln increased the brush border surface area through increased expression of structural and functional genes, thus inducing early brush border maturation during the peri-hatch period.

Key words: Intestinal brush border; In-ovo feeding; scanning electron microscopy; Gene expression

Expression of Chemerin in the magnum of chicken oviduct, perivitelline membranes, egg white and in extraembryonic annexes: a potential role in embryo development?

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In mammals, Chemerin (Chem), encoded by the gene Rarres2, is an adipokine involved in inflammation, adipogenesis, angiogenesis and energy metabolism. More recently, a role in reproductive functions has been reported. Chem exerts its physiological functions through the binding to three G protein-coupled receptors: the chemokine-like receptor 1 (CMKLR1), the G protein-coupled receptor 1 (GPR1), or the chemokine (C-C motif) receptor-like 2 (CCRL2). In chickens, we recently showed that the plasma concentration of Chem is associated with fattening but also to the hatchability of fertile eggs (Mellouk et al., 2018a and b). Moreover, we observed that Chem protein levels increase in adipose tissue, liver and pectoralis major specifically at hatching, and that this effect is associated with an increase in the embryo weight (Mellouk et al., 2018c). In this study, we first investigated the expression of Chem and its three receptors in the hen's oviduct (n=6 animals). By RTqPCR, western-blot and immunofluorescence by using home-made anti-chicken Chem antibodies, we found a strong expression of Chem and its receptors in the magnum and to a lesser extent in the infundibulum ($p<0.05$). These results led us to check Chem abundance in egg compartments (white, yolk and perivitelline membranes). By western-blot and specific ELISA assay, we showed a higher abundance of chemerin in egg white than in plasma ($p<0.05$). Chem was undetectable in egg yolk but strongly expressed in perivitelline membranes. After injecting different amounts of anti-chicken Chem antibody (0.1, 1 and 10 µg) in the albumen of fertilized eggs after 7 days of incubation (n=300 eggs injected in each condition, in six independent experiments), we observed significant embryonic mortality at day 12 (20% with 10 µg and 8% with 1µg , $p<0.05$). The level of embryo mortality in control experiments (after no injection or saline or mouse immunoglobulins), using the same experimental conditions was very low (less than 1%). Moreover, by immunoblot, we observed a strong expression of Chem in the various extraembryonic annexes (allantoic and amniotic membranes). Taken together, these results showed that Chem is strongly expressed in the magnum, and was identified in perivitelline membranes and egg white. Moreover, the negative impact of injection of Chem antibodies, on embryo viability suggests that this hormone is likely involved in embryo development. This work received a financial support from Région Centre Val de Loire (« PREVADI » project).

Key words: Hormone; Oviduct; Egg white; Embryo

Nutrient utilization of chicken embryos in a historical perspective

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Genetic selection and changes in management have improved production traits in different poultry species. Broiler chickens daily growth increased, while layer chickens increased egg production and both strains show a better feed conversion than in the past. These changes may also have affected growth and development of the chicken embryo and/or nutrient utilization throughout incubation. Growth and development of the avian embryo are largely dependent on nutrients deposited in the egg and these nutrients are fixed at oviposition. To investigate whether nutrient deposition, nutrient utilization and efficiency in nutrient use have been changed, six different scientific studies (1967-2016) of broiler and layer strains were compared. The total amount of solids in the initial egg was used to calculate the relative amount of solids that was found in the yolk-free body (YFB) and residual yolk (RY) at hatch, and in the external losses (including metabolic heat production and meconium). The efficiency to transfer solids from the initial egg to the YFB was expressed as a percentage and calculated by dividing the solids retained within the YFB by the solids used throughout incubation (formula efficiency=[(solid content YFB)/(solid content total egg – solid content RY)]*100%). The comparison of the six studies seems to show that the solid content of the RY at hatch has been decreased during the last 60 years and external losses have been slightly increased in this period. There was a lot of variation in the efficiency to transfer solids from the initial egg to the YFB, but there seems to be in general a slight increase in efficiency. These results indicate that modern chicken strains are able to utilize their nutrients more efficiently throughout incubation than strains used in the past, which is comparable to what has been found in the post-hatch period, although to a much smaller extent. This furthermore implicates that the small changes in metabolic heat production of modern broiler strains are probably influenced by the efficiency in which egg nutrients are utilized by the avian embryo. These changes are probably not only caused by genetic selection, but also by changes in incubation conditions and management.

Key words: Incubation; Chicken embryo; Nutrient utilization; Efficiency

2nd session:

Breeder management and multigenerational impacts

Impact of a Grape Seed Extract supplementation and a long storage period on egg quality, hatchability and post-hatching performance in broiler breeder hens

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Egg quality is influenced by the duration of storage that increases the oxidative stress. Grape seed extract (GSE), rich in phenolic compounds, are known to exert beneficial effects on the oxidative stress. Ours objectives were : 1. to determine the effect of long storage period on the eggs weight, early embryonic mortality (EEM), fertility parameters and on the quality of offspring and 2. to assess the potential protective effect of GSE on the storage period on the egg and chick quality. For this, 320 hens were divided in 4 groups: (A) control, (B) and (C) supplemented with 0.5 % and 1% of GSE, respectively since 4 weeks old and (D) supplementation with 1% GSE since the birth (D0). After artificial insemination, eggs from each group were collected for one week and the eggs from one day were stored during 2 weeks at 14°C and then incubated after. We performed three incubations. At E14, E17 (embryonic stages), DO (hatching day), D5 (5 days old) and D10 (10 days old), animals were weighted and and blood samples collected. The long period of storage decreased the weight of eggs at E14. It also affected the EEM and the hatchability. In A, B and D groups, the EEM was increased with the storage ($P < 0.05$) and the hatchability rate was reduced in A and B groups ($P < 0.05$), but unchanged in C and D groups as compared to A group. The storage had no effect on the fertility rate for the A, C and D groups but the rate was lower fo B group ($P < 0.0001$). Plasma uric acid level was lower in A group compared to stored groups at E14, however, at D0 and D5, the profile was reversed ($P < 0.005$). Plasma glucose level was lower in A group compared to stored groups at E21 and D10. At D10, the plasma triglycerides (TG) level was lower in C and D compared to A group. The storage did not modify the TG level. At E21, the weight of chicks was lower for A group and then, at D5 and D10, it was the opposite. For the stored groups, there was no difference for the diet, meaning that the storage period abolished the beneficial effect of the supplementation on the average daily gain of chicks.Taken together, 1% of GSE since 4 weeks-old reduced the damage induced by the long storage period on the EEM and it improved the hatchability rate (A=59 %, C=65%, D=66%). It reduced the weight of the chicks and abolished the increase of the average daily gain induced by GSE. These findings provide an insight into the potential effects of a GSE supplementation on the quality of eggs and chicks. This project was supported by PREVADI région Centre funding.

Key words: Grape seed extracts; Fertility; Broiler breeder hens; Duration of egg stora

Long-term effects of enrichment of hatching eggs with creatine on progeny

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The developing broiler embryo relies on nutrients deposited into the egg yolk and albumen by its maternal hen. In this study, GAA (guanidinoacetic acid), a precursor of creatine, was used as a feed additive in broiler breeder nutrition for enrichment of hatching eggs. This feed additive was used since creatine plays an important role in cellular energy metabolism. Creatine endogenous synthesis initiates in the kidneys, in which the AGAT enzyme generates a bond between arginine and glycine, creating GAA. In the liver, GAA is methylated by the GAMT enzyme, giving rise to creatine, which is then transferred through the bloodstream to tissues and cells by the creatine transporter, CRT.
The study aimed to evaluate the effects of broiler breeder supplementation with GAA on creatine levels in their eggs, expression of creatine synthesis and transporter genes in their progeny during the last days of incubation and the effects on their BW three weeks post-hatch.

The experiment consisted of Cobb broiler breeder hens that were raised in the Faculty of Agriculture (n=32) and divided into 2 groups: a control group which received standard feed and a GAA group which was given 0.15% GAA supplementation in their feed. After 11 weeks of feeding, eggs from both groups were collected at lay for examining creatine concentrations (n=8) and fertile eggs from each group were incubated (n=30). Tissues from progeny (small intestine, kidneys and liver) were sampled for Real-Time qPCR at E17, E19 and DOH and progeny BW were recorded at D20.

Results showed that creatine concentrations in GAA group eggs were significantly higher than control group eggs: a 57% increase in the albumen and a 99% increase in the yolk. GAA group progeny exhibited a significant decrease ($P<0.05$) in CRT expression at E19 and DOH, compared to control group progeny (1.4 and 1.3-fold, respectively). At DOH, AGAT expression in the kidneys and GAMT expression in the livers of GAA group progeny was significantly decreased (1.6-fold and 1.9, respectively), compared to control group progeny. At D20, BW of GAA group progeny were significantly higher by 5% ($P<0.05$) than control group progeny.

In conclusion, supplementation of broiler breeder hens with 0.15% GAA increased creatine deposition into the egg, subsequently affecting processes of creatine absorption and synthesis in the developing embryos and resulting in improved broiler performance through increased BW.

Key words: Creatine; GAA; Hatching egg; Gene expression

Effect of vitamin D source on embryo and early chicken development

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Vitamin D status of the breeders and offspring can influence muscle and bone development of broilers. The form of vitamin distributed to the animals, vitamin D3 or 25-OH-D3, can modulate their vitamin D status. The objective of this study was to evaluate the impact of breeder and broiler's vitamin D3 or 25-OH-D3 supplementation on embryonic, muscle and bone development. Two parental stock (PS) groups were supplemented respectively with only vitamin D3 (3,200 IU) or with 69-µg 25-OH-D3 + 440 IU vitamin D3 for 2 months. Eggs were characterized and resulting one day-old chicks were further fed experimental feeds (5,000 IU of vitamin D3 or 69-µg 25-OH-D3 + 2,240 IU vitamin D3). The assessed content of 25-OH-D3 was almost double in the egg yolk of PS fed 25-OH-D3, while the D3 content was more than 3 times higher in PS fed D3. These results validate the experiment and confirm that it is possible to enrich egg yolk in 25-OH-D3. Supplementation of 25-OH-D3 to PS did not influence egg quality parameters. After 10 days of incubation, the eggshell of PS fed 25-OH-D3 was heavier ($P<0.05$) but this difference did not persist. Interestingly, hatched-chicks from PS fed 25-OH-D3 exhibited 25-OH-D3 plasma content three times lower than those originated from PS fed D3. After hatching, chicks from 25-OH-D3 PS responded better to 25-OH-D3 supplementation and exhibited the highest 25-OH-D3 plasma content at day 6. Altogether, these data arise new questions about the ability of embryo to mobilize 25-OH-D3 in ovo and of chicks to potentiate dietary supplementation after hatch. During postnatal growth, the 25-OH-D3 plasma content was two to three times greater in chickens fed 25-OH-D3 than those fed D3. The impact of treatments on phenotypes was variable. At day 6, chicks from the 25-OH-D3 PS exhibited longer tibia ($P=0.02$) than those from the D3 PS, and chicks fed D3 exhibited larger tibia ($P=0.05$) than those fed 25-OH-D3. Such differences disappeared at days 21 and 36. During the whole period of growth, there was no difference in bone strength between treatments. At slaughter age, several interactions between PS and offspring diets were observed for body weight, abdominal fat and breast meat quality. Complementary molecular and histological analyzes are still running and will enable to decipher the mechanisms of action of vitamin D during early development in relation to the in ovo and post-hatch animal development.

Key words: Vitamin D; Embryo; Chick; Egg; Bone; Muscle

Impact of chemerin on sperm function and embryo development in broiler breeder chicken

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Chemerin is an adipokine that plays a key role in several biological processes such as adipogenesis and energy metabolism. Recent findings showed a negative correlation between plasma chemerin and some female fertility parameters including egg hatchability. So, we investigated the effects of chicken recombinant chemerin (ReCChem) on sperm of roosters since the fertility of these animals presents a real agronomic interest. By immunoblot and qPCR, we have shown that chemerin and its three receptors CMKLR1, CCRL2 and GPR1 were expressed within the testis from the embryo stages to the adulthood, with a lowest expression in adult testis. By using chicken specific ELISA and immunoblot, we observed that chemerin levels were significantly higher in blood than in seminal plasma. A qualitative analysis of roosters sperm highlighted a significant negative correlation between seminal plasma chemerin levels and the percentage of motility, progressive motility and the spermatozoa concentration. Furthermore, the percentage of static spermatozoa was positively correlated with chemerin concentration in seminal plasma. Finally, no correlation was found between chemerin concentrations in blood and seminal plasma. After in vitro experiments of fresh rooster sperm treated with ReCChem and/or with an anti-chicken CMKLR1 antibody and measurement of the massal motility of sperm under microscope, we showed that ReCChem inhibited sperm massal motility and this effect was abolished when sperm was pre-incubated with the specific anti-chicken CMKLR1 antibody. After In vivo experiments of fresh rooster sperm incubated with ReCChem and used for artificial insemination (AI), we observed a negative effect of chemerin on eggs fertility for the three first days after AI. Then eggs' fertility became identical between the tested conditions, suggesting a transitory negative effect of chemerin on sperm function. Moreover, chicks born from hens inseminated with sperm treated by ReCChem exhibited a lower body weight during their first 10 days of life. Taken together, seminal chemerin levels are negatively associated to the rooster fertility and chemerin produced locally by testis or male tract could negatively affect in vivo sperm quality through CMKLR1 in rooster. Moreover, chemerin levels in sperm could affect the growth of the chicks. Thus, the chemerin system is a negative regulator in male reproductive function in chicken. This projet is supported by the Région Centre ''PREVADI''.

Key words: Male fertility; Embryo development; Hormo

Monitoring of Chicken Astrovirus in broiler breeder flocks presenting low hatchability and progeny viability

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Enteric viruses are associated with production problems in commercial chickens, producing clinical signs such as stunting syndrome in broilers and low hatchability in broiler breeders (G*allus gallus*). In the last year in Brazil, there were problems of low hatchability within some breeding companies, registering embryonic mortality values of up to 70%, where the presence of enteric viruses such as CAstV was detected in embryo and digestive organs of stunting chicks. In these outbreaks, many of the chickens that hatched showed growth retardation, bristling of feathers and whitish colouring of feathers, showing signs of white chicks syndrome. As part of routine monitoring of CAstV, 346 pools of faeces samples were collected from 120 sheds from the bed litter. The samples were divided into 13 groups and were collected every week in subgroups of 25 to 29 pooled samples. Each pool was composed of ~100 g of faeces from each shed, totalling 4 sheds per pool. These pools were mixed and homogenized, and a small portion of ~1 g was separated for molecular analysis. After RNA extraction, a fragment of the CAstV genome was amplified by RT-PCR, according to the method described by Day, J.M. (2007). Out of 346 samples, 94 (27%) were positive for CAstV. Among the 94 positive samples, 29 RT-PCR products were randomly selected for sequencing by the Sanger method, confirming the presence of CAstV when we performed a specificity analysis of the nucleotide (nt) sequences with NCBI Blast. A similarity matrix showed that the 29 nt sequences shared 86%-100% similarity and that the deduced amino acids (aa) sequences shared 93%-100% similarity. When compared with sequences from the United States, the sequenced samples showed 84%-96% (nt) and 93%-99% (aa) similarity, whereas in comparison with samples from previous years in Brazil, they showed 84-95% (nt) and 85%-98% (aa) similarity. A phylogenetic tree clustered 12/29 sequences in a branch with a common ancestor for Italian and Croatian sequences, and the remaining 17/29 sequences were clustered in an isolated branch separate from even previous Brazilian sequences. Through this monitoring method, it was possible to relate the presence of the virus in the bed litter with the decrease in production parameters in the incubators and in new progeny performance. Further RT-PCR analysis confirmed the presence of CAstV in embryo and hatched chicks, demonstrating both ways of transmission, vertical and horizontal.

Key words: Astrovirus; Hatchability; Stunting-syndrome; RT-PC

Consequences of an antioxidant embryonic environment on broiler breeders reproductive activity

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Growth selection and feeding strategies have consequences on the reproductive activity. In case of overweight, the follicle hierarchy is disorganised (Hocking, 1993; Mellouk et al., 2018), causing internal ovulations and decreasing the egg production, fertility and hatchability (Robinson and Wilson, 1996). Unrestricted males present an early sexual maturity but in adulthood, sperm production decreases quickly (Robinson and Wilson, 1996). In order to better evaluate the consequence of genetic selection or feed additive on gonad development we assessed new indicators related to metabolism and kinetics of reproductive parameters on the same animals by using non-invasive tools such as medical imaging. The use of ultrasound and CT (Computerized Tomography) scan allowed us to follow some reproductive parameters in both males and females. By imaging analysis the number and the volume of yellow follicles and the ovarian fat volume could be quantified. The number of follicles was correlated with ovarian fat tissue ($r = 0.80$, $P < 0.001$) and with the body fat tissue ($r = 0.63$, $P < 0.01$). In male, the development of the testis increased rapidly from the time of light stimulation in an exponential manner and was detectable by CT-Scan at 24 week old, where the testis weight peaked, then the testicular volume gradual declined the following weeks.

Using these tools, we analyzed the development of chicks from mothers who had an antioxidant diet enriched with Grape Seed (GSE). Hence, the GSE-females (females exposed in embryonic stage) had an earlier puberty in comparison to female controls. However, at 34 weeks old, the GSE-females presented 18% less fat in the whole body and in ovary associated with a reduction in the number of yellow follicles (30% of GSE-hens had 6 yellow follicles compared to the 70% of control hens). Similarly at 30 weeks old, the GSE-mature chickens had a 13% reduction in the testis volume associated with a decrease in semen quality. These data show that the use of imaging tools makes possible the prediction of reproductive quality of animals. These measures could be performed in the context of genetic selection since these data are obtained in a non-invasive manner and consequently could be performed on animals in breeding.

Key words: Antioxidant; Testis; Semen quality; Fertility

Nutritional programming of hepatic metabolism in mule ducks

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The effects of maternal nutrition on offspring phenotypes have been mainly documented over the past years in mammals, and are now studied in poultry as well. We investigated the effects of a reduced level of dietary Methionine (Met) on laying performances of female common ducks (Anas platyrhynchos) and their impacts on the phenotype of their mule ducklings, obtained from an inter-generic crossbreeding with Muscovy drakes (Cairina moschata).A total of 60 female laying ducks were divided into 2 dietary treatments at 10 weeks of age. The restricted group received Met-restricted diets (R group) containing 0.25% of Met whereas the control group received control diets (C group) containing 0.40% of Met. The restriction was applied during the growing and laying periods, from 10 to 51 weeks of age. Neither the growth, nor the egg laying curve were affected by the methionine restriction. The fertility and hatching rates were also not affected. On the contrary, the total weight ($P < 0.001$), the albumen weight ($P < 0.001$) and the albumen percentage of dry matter ($P < 0.01$) were decreased for eggs laid by female breeders from the R group. Both male and female ducklings from the R group showed a reduced body weight at hatching ($P < 0.001$) and a tendency to an increased proportional liver weight ($P = 0.07$). Moreover, the maternal Met restriction modified plasma parameters in newborn ducklings regardless of sex: the alkaline phosphatase (ALP) and the alanine transaminase (ALT) activities were reduced ($P = 0.07$ and $P = 0.002$ respectively), the levels of glucose ($P = 0.03$) and triglycerides ($P = 0.01$) were higher whereas the level of free fatty acids decreased ($P = 0.01$). At the hepatic level, a study targeted on 170 genes of interest identified 51 differentially expressed genes (DEG). At 12 weeks of age, the animals from the R group showed decreased liver lipid level and abdominal fat weight ($P = 0.005$ and $P < 0.04$ respectively). Finally, at 14 weeks of age and after forced-feeding, the fatty liver weight was reduced in the R group ($P < 0.001$).In conclusion, the dietary restriction applied during gamete production, and the impoverished nutritional environment during embryonic development, may be involved in the changes observed in the hepatic and lipid metabolisms in ducklings. Finally, the impact of the nutritional programming is still observed at 14 weeks of age, after 12 days of force-feeding.

Key words: Duck; Nutritional programming; Methionine; Hepatic metabolism

Relation between fertility and insemination concentration dose after artificial insemination of cryopreserved chicken semen

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The relation between fertility and the insemination concentration dose (ID) of cryopreserved chicken semen was studied. Chicken semen was collected from commercial line cockerels housed in single cages and kept according to the standard management guidelines for chicken breeders. Ejaculates were pooled into semen samples and sperm quality was assessed soon after collection. Semen samples were processed for cryopreservation according the following steps: 1) dilution in pre-freezing modified Lake diluent supplemented with trehalose 0.1 M (LD); 2) equilibration at 4°C for 30 min; 3) further dilution in LD added with N-methylacetamide, 6% final concentration, and equilibration for 1 min; 4) loading into 0.25 ml French straws; 5) freezing on a rack floating over a nitrogen bath at 3 cm of height for 10 min; 6) transfer into cryotank for storage. Semen thawing was performed in ice-water bath at 5°C for 100 sec. During semen processing, the correct semen dilution was calculated to reach the following final sperm number per straw corresponding to different IDs: A) 250×106 sperm; B) 500×106 sperm; C) 750×106 sperm. One artificial insemination was performed using semen from one straw per hen. Laying hens (n=27) were divided into 3 groups receiving different IDs according to group A, B and C. Eggs were collected from the second day after AI for 10 days, set every 3/4 days and fertility recorded at candling after 7 days of artificial incubation. All clear eggs were open and true fertility recorded.The overall fertility value recorded in group A, B and C was 6, 8 and 11% respectively and a different fertile period was recorded according to the IDs. In hens receiving 250×106 sperm, fertile eggs were recorded only from day 2 to 4 and the highest fertility value, corresponding to 25%, was recorded on day 3. In contrast, in hens receiving 500×106 and 750×106 sperm, fertile eggs were recorded from day 2 to 10 and the highest fertility values recorded in group B and C were 17 and 20% respectively.In conclusion, the 250×106 sperm ID was suitable to assess in vivo fertility of cryopreserved chicken semen, even if the fertile period was limited to few days after artificial insemination. Increasing the ID to 500 and 750×106 sperm did not improve the fertility rate of cryopreserved chicken semen, but a longer fertile period was recorded.

Key words: Fertility; Insemination dose; Cryopreserved sperm; Chicken

3rd session:

Egg storage, preincubation conditions and incubation of commercial poultry

Effect of egg cooling rate after oviposition and SPIDES on embryonic development, and hatchability of long stored older flock eggs

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This study investigated the effect of the egg cooling profile of grandparent hatching eggs after oviposition and SPIDES on embryonic development and hatchability of fertile eggs. Hatching eggs from Ross female line grandparent flocks at 51 and 55 wk of age were used in Experiment 1 and 2, respectively. A total of 3,180 eggs that had been laid within a 15 minutes period were collected and then randomly assigned to two cooling profile groups with either control (CC) or slow cooling (SC) in each experiment. CC group eggs were directly transferred to storage room at 17°C, but SC group eggs were held in the holding room for 8 h at 27°C then sent to egg storage room. EST was cooled down to 24°C in 3h and 10h in CC and SC groups respectively. All eggs were stored for 14 days at 15°C and 75% RH. During storage, eggs were either held continuously at 15°C in the storage room (Control) or were subjected to a heat treatment regimen delivering 4 hours above 32°C, in a Petersime Re-Store machine at d 4 of storage (SPIDES). Some (30 embryos in each batch) of the eggs were opened after cooling process (0 d) and SPIDES (4 d) to determine the stage of the blastoderm. Each tray of 150 eggs was considered to be a replicate and there were 5 replicate trays per heat treatment in each cooling profile treatment. The eggs were randomly set in a single commercial incubator. Data from the 2 (cooling profile) x 2 (heat treatment) completely randomized design were subjected to analysis of variance. The SC vs. CC exhibited an EGK of 11.4 vs. 10.7 in Experiment 1 and EGK of 11.6 vs. 10.6 in Experiment 2 and the blastoderm development was significantly advanced by SC in both experiments ($P<0.05$). Blastoderm stages of embryos in the SPIDES treatment were more advanced ($P<0.05$) than those of the Control treatment (EGK 11.5 vs. 10.6 in Experiment 1 and EGK 11.7 vs. 10.6 in Experiment 2), as expected. Fertile hatchability was 87.6% and 85.7% in Experiment 1, and 83.4% and 80.3% in Experiment 2 in CC and SC groups respectively. In both experiments fertile hatchability decreased by slow cooling due to significantly increased early embryonic mortality ($P<0.05$). SPIDES exhibited numerically higher fertile hatchability (+1.7%, and +1.5% in Exp 1, and 2) than control in both experiments. It can be concluded that although both SC and SPIDES advanced the stage of blastoderm development similarly, hatchability was affected negatively by SC but beneficial by SPIDES, in the case of the old flocks.

Key words: Hatching eggs; Egg cooling profile; SPIDES; Embryonic development; Hatchability

Heat treatments during prolonged storage of eggs from young and old breeders

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Long periods of egg storage between laying and incubation cumulated with low qualitative flocks' ages degrade eggs quality. These parameters are inherent to breeders' availabilities and market demand, and cannot be always optimized by hatcheries. This project aims to test different factors during egg storage to compensate these critical but imponderables situations. In order to improve broilers health and welfare and limit the mortality during rearing, this study aims to increase chick robustness by use of two heat treatments during egg storage when the eggs came from one **Y**oung breeder flock (28 weeks of age) and one **O**ld breeder flock (59 weeks of age) and were stored for a long period (14 days).

9600 Y eggs and 9600 O eggs (ROSS 308) were stored according to three modalities: 1/"**C**": 11.5°C during all storage period as cold conditions to limit embryo cell death; 2/"**S**": two heats on the 6th and 10th days post-lay- each lasting 4 hours between 32 and 35°C, the rest of the time at 18°C, to make embryo reach robust development stage; 3/"**T**"; 18°C during all storage period as a control group, similar to commercial conditions. All groups of eggs within Y and within O were incubated in the same machine. Chicks were all reared in the same experimental farm. Embryo staging before incubation, hatchability, embryonic mortality, chick quality, broiler mortality and zootechnical performances up to slaughter were compared within groups of same breeder flock origin. This study showed that for Y and O, S accelerated embryonic development (stages EG&K 12.4 and 12.6 in SY and SO eggs vs. 10 in others groups, $p<0.01$), hatching rates were increased in C and S compared to T (for Y: 86% in T vs. 95% in S and in C; for O: 75% in T vs. 86% in S and 88% in C, X^2 test, $p<0.05$ for each comparison with T). C and S decreased embryonic mortality during storage and during incubation compared to the treatment T, and increased chick quality in terms of percentage of 1st graded chicks ($p<0.05$). Mortality during rearing was not significantly different between groups. T chicks were heavier than S and C at day 1 (for O), and than S only for Y ($p<0.05$). However there was no significant difference between groups on body weight after day 4. Feed intake was not different between groups.

Heat treatments for young and old breeder's eggs seem to compensate the negative effects of prolonged egg storage on hatching results even if differences on mortality or weight do not persist during all livestock.

Key words: Chick quality; Embryonic development; Mortality

Incubation temperature affects bursa of Fabricius histology of broiler chicks at hatch

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The bursa of Fabricius is an important lymphoid immune organ in young birds that contains follicles which produce B-cells. During incubation, embryo organ development is affected by eggshell temperature (EST). A constant EST of 37.8°C throughout incubation has been shown to result in most optimal organ development. However, based on recent studies, it can be hypothesized that a higher EST in wk 2 of incubation in combination with a lower EST in wk 3 of incubation might stimulate organ development, including bursal development.

To study this, 468 eggs of a prime Ross308 broiler breeder flock were incubated at different EST patterns in a 2x2 factorial arrangement. In wk 1 all eggs were incubated at 37.8°C EST. In wk 2, EST was either 37.8°C or 38.9°C. In wk 3, EST was either 37.8°C or 36.7°C. After 19.5d of incubation, every 6 h the incubators were opened to check whether chicks had hatched. From 12 chicks per treatment the bursa was collected within 6 h after emergence from the eggshell. Bursas were fixated in 4% formaldehyde with PBS for 48h and stored in 70% ethanol until processing. At processing, bursas were embedded in paraffin, sliced, and mounted on microscope slides. Slides were hematoxylin and eosin stained and number of follicles, follicle length, width, circumference, surface area, and cell density within follicles was assessed by using light microscopy.

Results showed that EST in wk 2 and EST in wk 3 tended to interact on follicle circumference ($p=0.10$). Follicle circumference was not affected by EST in wk 2 when EST in wk 3 was 36.7°C, but when EST in wk 3 was 37.8°C then a higher EST of 38.9°C in wk 2 tended to decrease follicle circumference compared to an EST of 37.8°C in wk 2. A higher EST in wk 2 of 38.9ºC resulted in a lower ($p=0.02$) cell density within follicles (39.2px) compared to an EST of 37.8ºC (46.0px). Additionally, a lower EST in wk 3 of 36.7ºC resulted in a higher ($p=0.01$) cell density within follicles (46.8px) compared to an EST of 37.8ºC (38.4px). Other variables did not differ between treatments ($p>0.10$).

In conclusion, this study has shown some evidence that incubation temperature patterns can affect bursa of Fabricius development of chicks at hatch. A higher EST in wk 2 seems detrimental for follicle circumference and cell density within follicles whilst a lower EST in wk 3 seems beneficial for cell density within follicles. Whether this might influence chicken immune responses in later life needs further investigation.

Key words: Incubation; Eggshell temperature; Broiler; Bursa

Evolution of incubation conditions for commercial poultry over the last 10 years and trends for the future

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Globally, broiler hatchability has improved slowly but steadily over the last 10 years at a rate of about 0.5% per year. This gain has been has been a result of improved breeder flock management, improved incubation techniques and genetic selection. Improvements in incubation techniques and technology have primarily focused on the requirements of the egg through measuring eggshell temperature and egg water loss. Major recent developments have focused on the hatching process and providing broiler chicks' early access to feed and water. Techniques such as providing food and water in the hatcher or hatching chicks directly into the chicken house are both being carried out successful in commercial poultry operations. While providing the optimal incubation environment has been shown to improve post-hatch performance, some research has also shown that short term changes to incubation temperature can result in specific beneficial modifications to post-hatch performance. However these studies have not yet resulted in changes to commercial practice.What does the future hold? Many of the current themes will continue to develop, in particular how incubation can affect post-hatch performance. Some studies have shown that light during incubation may have some important performance effects and this may lead to commercial benefits. Research on methods to sex in-ovo is starting to develop technologies that are successful and it is likely that these will be used in commercial hatcheries, particularly for laying chickens, in the near future. The drive to reduce the use of antibiotics in the poultry industry means that it is becoming more important to minimise contamination levels in the hatchery. Removing non-viable embryos at an early stage of incubation can significantly reduce the spread of microbiological agents during incubation and hatching and equipment to do this has now being developed and is likely to be more widely used in the future. Modern hatchery equipment continuously monitors the incubation environment and traps the data into databases. These databases can be connected to other databases for hatch results and broiler performance for analysis to determine the incubation parameters that impact results. In the future Big Data will become as important in that hatchery as it is in all other aspects of business.

Key words: Incubation; Poultry; Review; Hatching; Post-hatch performance

Transport of 18-day old hatching eggs - effects on physiology and behaviour in broiler chicken?

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Incubation, hatching, and raising of broiler chicken commonly takes places at separate facilities. Recently, hatching at the grow-out facility is tested, avoiding the transport of day-old chicks. Instead, eggs are transported on embryonic day 18 from the breeding to the grow-out facility, where they hatch on day 21.

Transport is a known stressor to animals. As the Hypothalamic–pituitary–adrenal (HPA)-axis becomes functional around embryonic day 14-16, it is conceivable that transport of eggs may lead to a stress response and increased production of corticosterone in the embryo. Exposure to prenatal stress may program the HPA axis in the long term, affecting the coping capacity of the individual and negatively impacting the further development of the chick. Moreover, malpositioning (the embryo turning towards the “wrong” end of the egg) of late-stage embryo seems to occur more often after transport of long durations, leading to hatching failure.

In this project we will investigate whether prolonged transport on day 18 has effects on the development of a slow growing broiler chicken strain. We measure the heart rate of embryos during transport and later hatching success of the chicks. We perform several established behavioural tests related to stress susceptibility, such as tonic immobility (TI) and open field (OF). Moreover we determine corticosterone concentrations in feathers at the end of the experiment.

We expect chicks that underwent longer prenatal transport to show stronger behavioural responses to stressful challenges, e.g., show a longer duration of TI, and higher levels of feather corticosterone compared to chicks that experienced short transport.

Key words: Welfare; Management; Stress; Corticosterone; Heart rate

4th session:

Egg quality, embryonic development and chick quality

Evolution of innate immunity components during incubation of the avian egg

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Successful avian reproduction relies upon the integrated defensive features of the calcareous eggshell, its acellular membranes (eggshell and vitelline membranes), as well as physicochemical characteristics of the egg white and the diverse antimicrobials that are distributed throughout the egg. These features are sequentially elaborated during egg formation within the hen reproductive tract, and act in concert to protect the egg and resist contamination of its precious contents, for successful development of the embryo. However, most elementary structures of the egg undergo dramatic modification during embryonic development. In parallel, egg white and yolk antimicrobials are assimilated by the embryo, while the acellular vitelline membrane that surrounds the yolk is rapidly degraded as the yolk sac expands onto the yolk. Moreover, the internal eggshell components are progressively disintegrated to provide calcium for embryonic growth and bone mineralization. Since chick innate immunity only matures after hatching, the primordial egg defenses are replaced by new protective and functional extraembryonic structures that are continuous with the embryonic tissues. These include the amniotic, allantoic sac (including the chorioallantoic membrane) and yolk sac. During incubation, they provide vital functions, such as predigestion of egg nutrients, respiratory exchange, mineral transfer from the eggshell to the embryo, and storage of metabolic/toxic wastes, until developing organs and tissues of the embryo become functional. In fact, most of these extraembryonic structures are discarded at hatching. The role of these membranes in protection of the embryo is not yet fully characterized, although they all cooperate in isolating the embryo from an aggressive physical and microbial environment. In addition, some of these vascularized structures are responsible for cellular and molecular innate immune responses that contribute to embryo protection. This review will present an overview of the different levels of these interconnected and dynamic egg defenses that assist the embryo throughout its development, beginning with the defenses of maternal origin, and describing the autonomous protective systems that subsequently develop within the incubated egg.

Key words: Chick embryo; Eggshell; Proteinaceous membranes; Chorioallantoic membrane; antimicrobials; Toll-like receptors; Innate immunity

Effect of female age and genotype on eggshell quality in ostrich females

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Problems related to egg quality and embryonic development are well described for most of the domestic species of birds. Ostrich females have a considerably longer productive life than other poultry species, making comparison for shell quality difficult. It is important to understand the eggshell characteristics of birds to ensure the successful hatching of healthy chicks. For this study 14 146 eggshells from a pair-bred ostrich flock were measured and analysed. The flock consisted of a total of 188 breedings pairs and the flock structure included different genotypes: South African Black (SAB), Zimbabwean Blue (ZB) and Kenyan Rednecks (KAR). The eggshell properties recorded were pore count, average pore diameter, total pore area of all the pore clusters in a given area and shell thickness. Shell thickness was measured after the membranes were removed and used to derive the permeability (defined as the ratio of pore area relative to shell thickness).

Systemic factors affecting eggshell quality included female age and genotype. Fixed effects considered included in general linear mixed model analyses were strain (SAB, ZB or KAR), female age (2 - 11 years), as well as year and season. Both pore count and permeability of eggs increased significantly in eggs of females older than 10 years. A significant increase in shell thickness was evident for eggs from females aged 2-year-old compared to females of 3 years and older and there was a marked reduction in shell thickness of eggs from females older than 10 years. SAB females had more pores per 2 cm2 than did the ZB phenotype, different combinations of SAB and ZB, as well as KAR. Pore count, permeability as well as shell thickness were compromised in eggs of older females, a finding that could partially explain the increased shell deaths in eggs produced by older females. It is thus important to consider all these aspects when planning breeding flock structure and incubation procedures.

Keywords: Eggshell pores; Permeability; Pore density; Shell thickness; Water los

Proteomic study of inner and outer hen egg vitelline membranes: insights into the biological functions of vitelline membrane layers

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The hen egg vitelline membrane (VM) is a proteinaceous membrane separating the egg white from the yolk. The VM is involved in fertilization, in early stages of embryogenesis but also in protection of the embryo. It consists of the inner layer (IVM) and the outer layer (OVM), that are in contact with the yolk and the egg white, respectively. These two layers are synthesized by distinct tissues namely the ovary/liver for IVM and the oviduct for OVM. About 140 different proteins were identified in the VM to date; however, the distribution of most of these proteins between IVM and OVM remains to be elucidated. The VM contains several ZP proteins that are likely to be involved in sperm-oocyte interactions during fertilization. VM is also crucial for embryonic development as it provides a substratum for embryonic and yolk sac expansion in early incubation. Moreover, VM is a physical barrier protecting the embryo from the egg white alkalinity and from potential microbial contaminations, and the high amount of antimicrobials in VM (lysozyme, AvBD11...) also contributes to the antimicrobial defense of the developing embryo. Thus, VM plays a key role, not only in fertilization, but also during incubation. The present study aims at determining the protein composition of IVM and OVM to identify the intrinsic putative functions of each VM layer. IVM and OVM were manually separated from the VM of freshly-laid eggs and proteins from these two layers were independently solubilized, and analyzed by SDS-PAGE and GeLC-MS/MS. More than 550 proteins were identified with two peptides in the whole VM. Around 440 and 380 proteins were identified in IVM and OVM, respectively. Preliminary data resulting from the functional annotation of proteins (gene ontology/bibliography) revealed the presence of many proteins putatively associated with cell adhesion and migration, in addition to proteins involved in fertilization and antimicrobial defense. Altogether, the data generated from this study will give new insights into the structure and composition of the chicken egg VM and will provide an integrative overview of the respective physiological functions of IVM and OVM. Next, it will be interesting to further study how storage conditions prior to incubation may alter the structural/molecular integrity of IVM and OVM, and consequently negatively or positively impact the revival of embryonic development during incubation.

Key words: Hen egg; Outer/inner vitelline membrane; Proteomics; Protein identification; Protein function

Yolk Sac Tissue development during incubation period

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The yolk sac tissue (YST) is an extra-embryonic tissue that envelopes the yolk content and has a critical role in embryonic development. Previous publications from our group showed that the YST functions as an intestine since it produces digestive enzymes and absorbs fat, amino acids and minerals. It also functions as a liver and gallbladder as it produces glycogen and bile acids. It was concluded that it serves as a metabolic organ, fulfilling the functions of various organs prior to their development by the embryo.

The aim of the current study was to examine the YST morphological changes along incubation by Scanning Electron microscope (SEM) and by light microscope, together with functional development, examined by gene expression. Hence, 100 Cobb500 fertile eggs were obtained and incubated, YST was sampled on embryonic days: E5, E7, E9, E11, E15, E18, E19, DOH. Several genes were selected in order to examine their expression along incubation: Thyroid hormones regulator genes (TG, TPO, TTR, DIO1,2), genes encoding for actin proteins responsible for microvilli formation (Villin, Ezrin, Espin), lipid metabolism and uptake genes (ApoA1, LRP2), glycogen synthase gene (GYS2), gluconeogenesis regulator gene (FBP1), and gene encoding for protein mediating the uptake of di-tripeptides (Pept1).

Observations by SEM revealed villi structures, comprised of endodermal epithelial cells (EEC's). Changes in morphology were evident. At E15, EEC's were bloated while at DOH they appeared shriveled. Measurements of EEC's perimeters showed a significant decrease from an average of 116.82 µm at E15 to 95.43 µm at DOH. In addition, the results showed that the EEC's contain microvilli on their apical membrane with an increase in their quantity and distribution towards hatch. All examined genes were expressed in a dynamic pattern. Towards mid incubation, there was an increase in expression of the thyroid regulator genes, followed by a decrease towards hatch. This suggests that the YST also takes part in thyroid metabolism. Results of the current study suggest that the increasing numbers of microvilli in the YST towards hatch is related to an increase in nutrient requirement by the embryo. It is expected that YST development and functionality will be affected by incubation temperatures (e.g. 1.5oc below/above the standard of 37.8oc) through changes of EEC's dimensions, microvilli localization and quantity and changes in YST gene expression.

Key words: Yolk Sac Tissue; Chicken Embryo; Incubation

Model-based monitoring of chicken embryo status during incubation

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Many incubation variables interact with embryonic development. Often such incubation variables (e.g. eggshell and micro-environmental temperatures) have major influences and are associated with, for example, hatchability results and chick quality on group level. However, little is known on the interaction of such variables at individual level (e.g. Romanini et al., 2013). In this study, an individualised model-based monitoring technique is developed to detect and predict the individual progress of embryo development during the incubation of chicken eggs. The developed technique is based on measured eggshell temperature (TEgg) and micro-environmental air temperature (TAir). The three main objectives of this study were: i) to quantify the relation between eggshell temperature and the local environmental temperature based on individual first order discrete-time transfer function models. ii) To detect (i.e. monitor) different model-based milestones that can be related with the biological processes of the individual embryo development. iii) To use the individual information obtained by the detected embryo development milestones to make predictions of hatch time.

The results showed that five different milestones in the development of individual broiler embryos can be detected: i) transition from endothermic to exothermic; ii) plateau phase of embryonic metabolic heat production; iii) the start of pulmonary respiration from the breaking of the internal egg air cell (internal pipping); iv) the completion of the diffusive respiration via the chorioallantoic membrane with embryos pipping the egg shell externally (external pipping) v) the completion of breaking the egg and the emergency from the shell (hatch). In addition, we could make individualised prediction of the hatch day after detection of milestone ii, the plateau phase on embryo heat production (ROC AUC = 0.91). This result can be interpreted as an early biological sign of the embryo preparing itself in terms of metabolic energy saving to progress towards the hatching phase. This individualised monitoring approach could be an added value for individualised prediction of hatching and for other experimental designs and studies, where the developmental stage of the embryo is relevant. Moreover, this individualised model-based monitoring approach could also open possibilities in future studies for the development of more precise control systems for synchronised embryo development and uniform hatching.

Key words : Individual model-based monitoring; Developmental milestones; Discrete time transfer functions; Embryonic development; Eggshell temperature; Hatch time

Effect of egg weight loss and hatch time on chick yield and first week post-hatch performance

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This study investigated the effects of egg weight loss (EWL) and hatch time (HT) on chick yield and, 7d post-hatch performance. Broiler hatching eggs were obtained from a commercial flock of Ross 308 at 40 wk. Eggs (64 g ± 2 g) were numbered and weighed individually before set and during transfer (d19) to determine EWL. At transfer time, eggs were classified into three EWL groups: Low (8.0 % - 10.8 %), Standard (11.8 % - 12.2 %) and High (13.4 % - 15.4%) and sent to an on farm hatching system. Chicks were individually identified by neck tag at hatch and twice weighted at 510h (take-off) of incubation to determine the chick yield (CY). The hatching process was divided into three time periods: Early (475 h - 487 h), Middle (491 h - 493 h), and Late (498 h - 509 h). A total of 810 chicks were raised up to 7d of age when Body Weight (BW), Feed Consumption (FC) and Mortality (M) were recorded. Data were analyzed using a factorial random design of 3 EWL x 3 HT treatments.

Averages CY were 69.2 %, 67.9 %, and 66.0 % at 510 h for Low, Standard, and High EWL groups, respectively. In the same time, chick yield of Early, Middle, and Late hatched chicks was average 66.0%, 68.1%, and 69.1%, respectively. CY was significantly affected by both EWL and HT treatments ($P<0.05$). BW was greater in Low EWL (44.3 g) compared to Standard (43.5 g) and High (42.2 g) at 510 h ($P<0.05$) but this advantage disappeared by 7d and there was no significant difference in BW and FC among EWL groups at 7d. Late HT exhibited the greatest BW at 510 and 518 h, and the lowest BW and FC at 7d. ($P<0.05$). Mortality was not affected by EWL or HT. Combined treatment groups (Low EWL-Late HT; Standard EWL-Middle HT; High EWL-Early HT) had similar CY at 510h but differed in BW and FC at 7d ($P<0.05$). This result of this study demonstrated that either EWL or HT affects CY, with only HT having a seven days long effect on performance.

Keywords: Egg weight loss; Hatch time; Chick yield; Body weight; Feed consumption

From eggshell decalcification to skeleton mineralization of chicken embryos during incubation

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In oviparous species, the egg contains protective systems and provides energy and nutrients that support the development of the embryo until hatching. The calcification of the chicken skeleton relies on two main mineral sources: the yolk during the first half of incubation, and the eggshell, thereafter. The mineral/calcium transport from the eggshell to the embryo is achieved by the chorioallantoic membrane (CAM), which develops in close contact with the inner eggshell from day 5 of incubation onwards. However, the molecular actors associated with the function of this extra-embryonic membrane are still poorly characterized. The objective of this study is to obtain basic data to decipher the role of the CAM in the mobilization of eggshell minerals and in subsequent mineralization of the embryonic skeleton.

Sixty eggs (64.1±1.8g) from Rhodes Island laying hens were incubated for 12 or 16 days. At each stage, egg weight as well as various eggshell quality parameters were measured. Eggshell strength, weight and thickness all decreased during incubation, which validates the model. Currently, we are analyzing eggshells for changes in their mineral content (calcium, potassium, phosphorus, etc.) and have stained bones and cartilage of each embryo to monitor the kinetics of skeleton mineralization. We also collected the CAM to study the expression of mineral transporter genes. Data collection and statistical analyses of the results are in progress in order to assess stage-dependent changes.

We believe that this study will provide insight into the role of the CAM in mineral metabolism during chicken embryonic development, and help to identify molecular markers to explain post-hatch dysfunctions linked to impairment of bone integrity/structure in certain chicken strains. Considering that intensive genetic selection of broiler breeders and laying hens for specific performance traits (meat and egg production) has resulted in considerable differences in growth and in chicken intrinsic metabolism, we hypothesize that the kinetics of bone mineralization during embryonic development will exhibit strain-dependent differences. To further test this hypothesis, next studies will evaluate fast-growing, and slow growing broiler strains to compare molecular and phenotypic traits associated with mineral metabolism with those of Rhodes Island laying hens.

Key words: Chicken; Eggshell; Minerals; Embryo; Chorioallantoic membrane; Skeleton

Involvement of two-pore calcium channels in the regulation of the cardiac activity in the early stages of chick embryo development

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Calcium homeostasis influences the cardiovascular function in the developing chick embryo. It is know that calcium is a critical regulator of cardiac myocyte function as it links the electrical signals that pervade the heart and contraction of the myocytes to propel blood. In this process, calcium channels are the central players in the cardiomyocytes calcium signaling. We focused on the role of the two-pore calcium channels in the spontaneous rhythmic contractions of the chick embryonic heart. These channels are localized in endolysosomal vesicles and activated by the second messenger NAADP.

The aim of this study was to find out how the blocking of two-pore calcium channels will affect spontaneous rhythmic cardiac activity at the early developmental stage of chick embryo before the formation of embryonic hormonal or neuronal heart control. A selective antagonist of two-pore calcium channels trans-NED19, which is a structural analogue of NAADP, was used for the studies. The experiments were carried out on the spontaneously beating heart isolated from Day 4 embryo. The video recording of the isolated heart, transferred in a chamber filled with Hanks solution, was used, and then trans-NED19 was added to the solution at different concentrations. The videos were reviewed and analyzed using Danio Scope (Noldus, The Netherlands) computer software. In the control, the heart rate (HR) was stable and averaged about 111 bpm (N=14). Trans-NED19 (final concentration 10 μM) caused an inhibitory effect, and the HR decreased by 30 %. When the trans-NED19 concentration was increased by two times (20 μM) the HR decrease by about 42 % of the value in the control and it did not significantly differ from the effect of 10 μM. The HR completely restored by washing in a Hanks solution.

Our data suggest that two-pore calcium channels are involved in the cardiac spontaneous activity in the early developmental stage chick embryo. The absence of a significant difference in the effect of trans-NED19 on HR at 10 μM and 20 μM concentrations may indicate saturation of NAADP binding sites by this antagonist. We can assume that about 40% of the heart rate at this stage of chick development is associated with the mobilization of calcium from endolysosomes through the two-pore calcium channels activated by NAADP.

The work was conducted under the IDB RAS Government basic research program, № 0108-2018-0002 and 0108-2018-0003 and partially supported by RFBR grant №17-04-01267.

Key words: Two-pore calcium channels; Heart; Early chick embry

CrystalEgg-The world's first embryonic monitoring system

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Founded in 2015, LIVEgg™ is a young, dynamic and innovative biotech company. LIVEgg combines the deep knowledge and experience of its parent company BARAM GROUP, semi-integration poultry growing group, with its own hi-scale engineering and biological R&D capabilities to develop breakthrough-growing solutions for the poultry industry

LIVEgg developed an embryo monitoring system – CrystalEgg™ that in noninvasive ways provides on-line information of embryo development, and can detect many embryonic and physiological signals enabling the hatchery to closely watch the embryo.

The information acquired from the collected signals, enables the user, to guide the incubation procedure with extreme accuracy, get the optimal pull time and chick numbers, and control the development so the embryo does not suffer from any out of bound incubation parameters, hence receiving the best product and maximizing the breed potential. An additional application which is being investigated is predicting the precise timing of in-Ovo vaccination.

As modern broiler development is continuously improving, and any detour of growing goals is costly, CrystalEgg™ will enable the broiler to meet its top potential.

Sub optimal embryonic temperature, has been found to influence chick yield, chick mortality, impair intestinal growth and damage absorption, influence Heart size, impairing Cardiovascular development and function, and muscle growth affecting Carcass yield, and many other issues.
Present and Future Applications

- CrystalEgg™: LIVEgg launched its commercial flagship application: CrystalEgg – the World's first embryo monitoring system – which helps drive up profitability by providing the hatchery management team with real-time knowledge regarding the development stage and condition of the embryos within the incubator.
- ChickMale Saver™: scheduled for launching during 2020. ChickMale Saver™ is positioned to become the world's only non-invasive gender recognition system.

LIVEgg presentation will show the ability of CrystalEgg to bring a new set of information for the hatching process using two case studies: COBB and ROSS eggs.
Examining the different pace of embryonic development between the two breeds show the correlation between embryonic developmental stage and hatch window-D.O.C. uniformity.

Key words: Noninvasive; Embryo; Monitoring; LIVEgg; CrystalEgg; ChickMale saver; Embryonic signals; In-Ovo vaccination

Embryonic mortality of chicken embryos in a prenatal stage

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The aim of the study was to evaluate the effect of age of hen on reproductive indicators (fertility, hatchability and embryonic mortality) in ISABROWN breeders (IB) (Experiment 1). Breeders age of IB in Experiment 1 was 34, 56 and 58 weeks (w) of age. Another aim of the study was to detect the relation between the shell quality and the embryonic mortality in eggs from IB hens of 34w of age and of BOVANS GOLDINE (BG) hens of 38w of age (Experiment 2). The shell was monitored for subsequent attributes: shell strength, percentage of shell from the weight of the egg, shell thickness and relative conductance of the shell.

Experiment 1:

In IB of 34w of age fertility was significantly lower ($P<0.05$) than in IB of 56 and 58w of age. The hatchability from fertilized eggs was the highest ($P<0.05$) at 34w of age (84.4%) but was significantly lower ($P<0.05$) in eggs from 56w hens (78.0%) and of 58w of age (69.4%). Also, the hatch of fertile eggs (HOF) significantly declined ($P<0.05$) with age.

Experiment 2:

The embryonic mortality was significantly higher ($P<0.05$) in IB than in BG in all three parts of incubation. The fertility in IB at age of 34 weeks was 93.7% that was significantly lower ($P<0.05$) than in BG at age of 38 weeks where the fertility was 98.3%. The hatchability of fertilized eggs was significantly higher in BG (85.5%; $P<0.05$) than in IB (84.4%). Also, the HOF was significantly higher ($P<0.05$) in BG (84.0%) than in IB (79.1%). The embryonic mortality was significantly higher ($P<0.05$) in IB in all thirds of incubation. There was not significant difference ($P>0.05$) in hatching egg weight between BG and IB. Significant difference was not found ($P>0.05$) in percentage of shell from the weight of egg, shell thickness neither relative conductance of the shell. Only the shell strength was significantly higher ($P<0.05$) in BG than In IB.

With the increasing breeders age is HOF evidently decreased especially due to increasing embryonic mortality in the second week of incubation. In this study the strength of eggshell was positively associated with hatchability.

Key words: Hatchability; Shell quality; Fertility

5th session:

Epigenetics and long-term effects of perinatal environment and early life experience

Transgenerational epigenetic inheritance in birds

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Epigenetics involves accessory chemical modifications of the DNA that can i) regulate gene expression and ii) be maintained after mitotic events. Epigenetic mechanisms account for a portion of the variability of complex traits linked to interactions with the environment. Additionally, recent research has shown that epigenetic mechanism are also involved in non-Mendelian inheritance. From these observations, the concept of Transgenerational Epigenetic Inheritance has emerged. The mechanism involves direct or indirect exposure of the germ line epigenome, which generates disturbances that can affect the phenotype of descendants, i.e. unexposed individuals of subsequent generations. The germ line plays a fundamental role in this transgenerational process because it will transmit acquired epigenetic modifications between generations. Epigenetic modifications in the germ line take place during developmentally sensitive periods undergoing major DNA methylation reprogramming. In recent years, a growing number of studies have revealed that epigenetic modifications can be transmitted across generations in several animal species. Numerous studies have demonstrated inter- or multi-generational effects of changing environment in birds, but very few have shown epigenetic transgenerational inheritance. In this presentation I will delve into the concept of transgenerational epigenetic inheritance in animals, providing key examples for many species, and I will describe the current state-of-the-art in birds. I will place a special focus on chickens and the impact of early stressors on their behavior. I will underline the advantages and drawbacks of studying transgenerational epigenetic inheritance in birds and explore future directions.

Key words: Epigenetics; Non-Mendelian Inheritance; Avian; Birds; Chickens; Transgenerational; DNA methylation

Early-life epigenetic changes along the corticotropin-releasing hormone (CRH) gene influence resilience or vulnerability to heat stress later in life

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Stressful events in early life might lead to stress resilience or vulnerability, depending on an adjustable stress-response set-point, which can be altered during postnatal sensory development and involves epigenetic regulation of corticotropin-releasing hormone (CRH). During the critical developmental period (CDP) of thermal-control establishment 3-day-old chicks were conditioned at either moderate (36ºC) or acute (40ºC) heat conditioning for 24 hours. One week later (10-days of age) these chicks were challenged under moderate heat stress (36ºC) for 24 hours. On both days 3 and 10, chicks were sacrificed, the hypothalamic paraventricular nucleus (PVN) was dissected, and mRNA, and DNA were extracted for gene expression and epigenetic analysis including methylation analysis by sequencing and histone post translational modifications and protein-DNA interaction by Chromatin immunoprecipitation. Heat stress was found to affect both body temperature and expression of CRH in the PVN. Both increased during heat challenge in chicks that were trained to be vulnerable to heat, whereas they decreased in chicks that were trained to be resilient. Accordingly, DNA CpG methylation (5mC) and hydroxymethylation (5hmC) at the CRH intron, which we found to serve as a repressor element, displayed low 5mc% alongside high 5hmc% in resilient chicks, and high 5mc% with low 5hmc% in vulnerable ones. RE1-silencing transcription factor (REST), which has a binding site on this intron, bound abundantly during acute heat stress and was nearly absent during moderate stress, restricting repression by the repressor element, and thus activating CRH gene transcription. Furthermore, REST assembled into a protein complex with TET3, which bound directly to the CRH gene. Finally, the adjacent histone recruited the histone acetylation enzyme GCN5 to this complex, which increased H3K27 acetylation during harsh, but not moderate heat conditioning. We conclude that an epigenetic mechanism involving both post-translational histone modification and DNA methylation in a regulatory segment of CRH is involved in determining a resilient or vulnerable response to stress later in life.

Key words: Epigenetics; Stress; Heat conditioning; CRH

Long-lasting effects of perinatal temperature manipulations on hypothalamic mechanisms are age and sex-specific

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In precocial birds the hatching phase is a critical period in which perinatal temperature manipulations (PTM) induces long-lasting changes in physiological peripheral and central nervous mechanisms. This review focuses on own research projects on influence of chronic and short-term PTM on the hypothalamic microstructure controlling body temperature, feed intake, body weight and metabolism in terms of age and sex dependence. All experiments were carried out in brain slices containing the preoptic area of the anterior hypothalamus (PO/AH) or the Nucleus infundibuli hypothalami of the ventrobasal hypothalamus (NI/VH). In Muscovy ducks aged between embryonic day (E) 28 and day (D) 10 post-hatching, the neuronal thermosensitivity of the PO/AH and its modification by chronic PTM (35°C; 38.5°C; control 37.5°C) were investigated using extracellular recording. During early ontogeny (E28-D5) the PO/AH is characterized by a high cold and low warm sensitivity (up to 30% and 5%, respectively). Between D5 and D10 a strong qualitative change occurs towards the "adult" neuronal thermosensitivity, which is characterized by high warm and low cold sensitivity (D10 15% warm and 14% cold sensitivity). PTM related modifications show also a clear age-dependent pattern starting with proximate non-adaptive changes up to a clear incubation temperature dependent and proximate adaptive modification at D10 (cold-incubation increased warm sensitivity and decreased cold sensitivity and warm-incubation induced opposite modifications, $p<0.05$). The NI/VH contains orexigenic neurons expressing neuropeptide Y (NPY) and anorexigenic neurons expressing proopiomelanocortin (POMC), which are also key player in stress response control. In female and male broiler chickens effects of short-term PTM (+ 1°C, 2 hrs/day) on neuronal NPY expression were investigated on D35 using immunohistochemistry. Analysis of 365 slices results in a significant ($p<0.05$) decrease in NPY expression exclusively in males. It is corresponding to changes in thyroid hormones (T3/T4) and performance. Females show no or only tendential changes in the same parameters. Studies on POMC gene expression in three weeks old normal incubated laying type chickens, showing correlation of methylation pattern and gene expression along with sex-specific differences ($p=0.03$).In conclusion, PTM induced lasting changes in the microstructure of the hypothalamic neuronal network are obviously sex-specific and follow ontogenetic rules.

Key words: Hypothalamus; Preoptic area; Nucleus infundibuli; Neuronal thermosensitivity; NPY; POMC

Early experience affects zebra finch adult thermoregulation in the heat

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Experiences encountered early in life have profound effects on individuals' phenotype, by shaping developmental processes. Whilst the impact of early nutritional and endocrine environments on development has been intensively studied, the potential impact of sound was only recently discovered. In the zebra finch, an arid-adapted Australian passerine, we showed that parents emit a peculiar vocalisation during late incubation at high ambient temperatures. Both in the wild, and under controlled thermal conditions in the lab, parental calling is specifically triggered by high air temperatures, and interestingly, is predicted by individuals' body mass. Using playbacks in incubators, we showed that exposure of embryos to this call alone adaptively alters subsequent nestling growth in response to nest temperature, and influences individuals´ thermal preferences as adults. Most remarkably, we also show that early acoustic and thermal experiences affects individual thermoregulation at adulthood. Specifically, prenatal acoustic experience and post-natal nest temperature both influenced the body temperature, evaporative water loss, and resting metabolic rate of adults (n=44) measured in an open flow-through respirometry system. These effects specifically occurred at the highest air temperature birds were exposed to (42-44°C). Together, our results demonstrate that the effect of the prenatal acoustic environment on development is considerably greater than currently acknowledged. Our findings also shed light on a novel mechanism for thermal adaptation, which may be relevant across avian taxa, given the widespread occurrence of prenatal acoustic communication in birds, including in poultry.

Key words: Thermoregulation; Heat adaptation; Prenatal acoustic communication; Developmental programming; Maternal effects; Developmental plasticity

Evidence for epigenetic reprogramming in response to acoustic signals in zebra finch eggs

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Growing evidence suggests that developing birds sense acoustic cues from the environment, even while in the egg. Zebra finches provide a striking new example: at high ambient temperatures and in the presence of eggs, nesting parents produce an "incubation call", and embryos exposed in the egg to this call later develop with better adaptation to hot weather (Mariette & Buchanan, Science, 2016). These adaptations include altered growth rates and thermal sensitivities – traits of interest to poultry producers. Here, we report on ongoing experiments to test the hypothesis that incubation calls trigger an epigenetic response in the embryo, ultimately leading to adaptive developmental reprogramming. Eggs (n = 40) reared in an incubator were exposed for three days to incubation or control calls, to test for differences in gene expression linked to progression of developmental reprogramming. By RNA sequencing analysis (RNAseq) of whole brain, we see evidence for differential expression of ~10 genes in the eggs exposed to incubation calls. We are currently mapping the anatomical expression of these genes and replicating the experiment in a new set of embryos. These experiments provide initial support for the hypothesis that, despite their altricial development, zebra finch embryos respond to acoustic signals with changes in gene expression that may have lasting effects on growth and temperature sensitivity.

Key words: Embryo; Gene expression; Epigenetic; Reprogramming; Songbird; Brain

Early rearing conditions in chicken: effects on cognition and stress response

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Intact cognitive ability as well as the adaptive capacity to deal with varying levels of fear and stress are essential for animals to thrive in farm settings. In mammals, it is well established that, for instance, impoverished environments or malnutrition can lead to underdeveloped cognition and elevated stress responses in later life. Effects of the social and physical environment during rearing on cognition and stress response in pullets, and later in adult layer hens, is an understudied area. To study these factors, we first established and/or adapted several tests for measuring cognition, sociality and fear in chicken, including the chicken holeboard for short and long-term spatial memory; the Y-maze for sociality or for food reward; and the voluntary human approach test. We also established a protocol for measuring corticosterone in both primary wing feathers and in down as a long-term measure of stress.

We used these techniques in a series of experiments in layer chickens to study effects of stocking density during rearing (von Eugen et al, Animals 2019, doi:10.3390/ani9020053), rearing with or without a broody hen (Angevaare et al 2019, http://dx.doi.org/10.1016/j.applanim.2012.05.004; Hewlett et al, in prep), rearing with or without a dark brooder (Nordquist et al, submitted), and effects of genetic background on early life development (Nordquist et al, AABS 2011, https://doi.org/10.1016/j.applanim.2011.02.008). We also examined effects of housing single- or mixed-sex groups in broilers using these same tests and techniques (de Leeuw et al, ISAE Benelux Proceedings, 2016). We showed that overcrowding during rearing elevates feather cortisol and increases anxious behavior, and that (lack of) maternal care had surprisingly little effect on cognitive development in two separate studies. Dark brooder rearing lowered feather pecking and cannibalism levels, while having no effect on corticosterone in feathers, which implies that the dark brooders help prevent the development of injurious behaviors while not affecting the HPA axis.

Together these results emphasize the importance of the rearing period on later cognitive and stress-response development in layer hen, and the need for further research in this area. Many questions remain, including effects of genetic strain on acceptance of maternal care, and effects of even earlier events (i.e. suboptimal incubation conditions or transportation prior to hatch) on later stress responses and cognition.

Key words: Rearing; Behavior; Corticosterone; Cognition; Sociality; Fear

Effect of chick holding temperature and provision of a hatchling supplement on body temperature, blood glucose, residual yolk sac utilization and livability

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The objective of this study was to observe chicks held under different holding temperatures through a simulated 72 hour journey. Ross 308 parent stock chicks from a single farm were incubated across 3 setter/hatchers. Temperatures were adjusted to achieve a constant 37.8°C eggshell temperature. On removal from the hatcher, the residual yolk sac weight averaged 5.23g (12.03% of the average chick body weight). Chicks were counted, graded and randomised into three groups of chicks, each held in 26 export quality cardboard boxes holding 68 chicks per box. The hatchers were set up to mimic chick delivery trucks, with three different temperature regimens. The control (OPT) treatment delivered an air temperature in the chick box of 30°C. The warmer treatment (WARM) held the temperature at 36°C and the cooler (COOL) treatment delivered 24°C. Wet bulb set points were adjusted to deliver 60% relative humidity in each machine. Sample chicks were removed at 0, 24, 48, 60 and 72 hours to measure vent temperature, body and residual yolk sac weight. Daily mortality was removed and recorded.After 72 hours, chicks were placed in trial pens (6 replicates of 45 chicks per treatment) and were grown to 7 days. Daily mortality was recorded.The average vent temperature at hatch was 39.97°C. Over the first day, the chick temperature in the WARM and COOL treatments diverged from the OPT, but behavioural modifications (panting and huddling) kept them within target (39.44 to 40.56°C) for chick comfort at 24 hours. By 48 hours, the chicks in the cool treatment were below target for comfort, and their temperatures continued to drop. The OPT and WARM treatments stayed within target to 72 hours. Temperature did not affect the rate at which the residual yolk was used. However, the average weight loss of the OPT chicks was 13.7%, compared with 15.4% for the COOL and 19.2% for the WARM treatment. During the holding period, mortalities from the OPT, WARM and COOL treatments were 0.0%, 0.2% and 1.7%. First week mortalities in the broiler house were 4.4%, 6.3%, 21.9%. Chicks can be held for 72 hours in an air temperature of 30°C and survive well. Cooler temperatures were associated with raised mortality.

Key words: Holding temperature; Supplement; Body temperature; Residual yolk; Livability

Effect of feed and water deprivation during transport on post-hatch performance of early fed chicks

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Many chicks have immediate access to feed and water in the hatcher nowadays, while they are deprived from feed and water during transport to the broiler house afterwards. This is suggested to have a negative effect on post-hatch performance. In the current trial we investigated how detrimental a period of feed deprivation is to post-hatch performance of chicks that had immediate access to feed and water during the hatching period.

In the hatcher, chicks had immediate access to feed and water or had no access to feed and water. After pull, chicks had immediate access to feed and water or were deprived for a period of 27 hours, simulating a prolonged transport period. The treatments were EF-TF (Early Fed-Transport Fed); D-TF (Delayed Fed-Transport Fed); EF-TD (Early Fed-Transport Deprived); D-TD (Delayed Fed-Transport Deprived). Ross 308 chicks of a breeder flock of 55 weeks were used. Each treatment had 6 replicate pens containing 20 male chicks. Body weight (BW) was determined per pen at d0, d1 (end of deprived period for TD treatments), d7, d14, d28, and d35. Residual feed was weighed per pen at d7, d14, d28, and d35. ADG, FI, and FCR were calculated. Mortality was recorded daily.

At pull time, BW was 6.6g higher for immediate fed chicks than for delayed fed chicks ($P<0.001$). At d1 and d7, an interaction between feed and water in the hatcher and during the transport period was found ($P=0.01$ and $P=0.03$, respectively). At d1, BW of all treatments differed from each other: 68.1g for EF-TF, 58.5g for D-TF, 48.3g for EF-TD, and 43.1g for D-TD. At d7, EF-TF had a higher BW than D-TD, while EF-TD and EF-TF were intermediate. ADG between d1-d7 of immediate fed chicks in the hatcher was 3.1g higher than of delayed chicks. This resulted in equal BWs at d7 for treatments EF-TD and D-TF despite the difference of 10.2g at d1.

A main effect of immediate feed and water in the hatcher was found on BW at d35 ($\Delta=74g$; $P=0.02$), while no main effect was found of feed and water during the transport period ($P=0.07$). For DFI between d0-d35 a positive effect of feed and water in the hatcher and during the transport period was found ($\Delta=3g$; $P=0.01$ for both main effects). FCR and mortality were not affected by the treatments ($P>0.19$).

In conclusion, immediate feed and water in the hatcher increased BW at d35 in comparison to no feed and water in the hatcher, while no effect on BW at d35 was found for feed and water or no feed and water during a period of 27 hours after leaving the hatcher (transport period).

Key words: Broilers; Early feeding; Feed deprivation; Performance

Long-lasting metabolomic fecal signatures of negative postnatal events in chicks

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Negative experiences in early life can induce long-lasting effects on the welfare, health, and performance of farm animals. A delayed placement of chicks in poultry houses has negative effects on their performance. Based on this observation, the metabolites from the feces of 12-day-old chickens were screened for early markers of response to negative events using gas-chromatography and liquid-chromatography coupled with mass spectrometry (GC-MS, LC-HRMS). The fecal metabolome from 12-day-old chicks having experienced an optimal (control) or delayed placement (delayed) were recorded by GC-MS and LC-HRMS in two genotypes from two experiments. From both experiments, 25 and 35 metabolites, respectively explaining 81% and 45% of the difference between delayed and control chickens, were identified by orthogonal partial least-squares discriminant analysis from LC-HRMS and GC-MS profiling. This study showed that the fecal metabolome was durably influenced by the postnatal events experienced by chicks. The model highlighted persisting differences in metabolites involved in adaptive response, energy metabolism, and microbiota composition between delayed and control chicks in response to the negative postnatal experience.

The sets of molecules identified will be useful to better understand the chicks' response to negative events over time and to define stress or adaptation biomarkers. They will provide tools for assessing potential innovative practices designed for improving chicken health and welfare.

Funding: Integrated Management of Animal Health metaprogram of INRA for the "GISA-WHELP" project and network http://www.gisa.inra.fr/en.

Key words: Biomarker; Chick; Feces; GC-MS; LC-MS; Negative postnatal event

The effect of various eggshell temperature patterns on hatchability, chick quality, and post hatch performance

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A constant eggshell temperature (EST) of 37.8°C has been shown to result in good hatchability and chick quality, but some variations in EST may be even more beneficial. For example, colder EST (36.7°C) in the final days of incubation and brief peaks or drops in temperature have been suggested to improve organ development at hatching and post hatch performance. 3 EST patterns were applied: Control (constant 37.8°C), Cold (37.8°C till embryonic day (E)16, then a gradual decrease to 36.7°C on E18), and Spiking (like Cold, but briefly spiking to 40.0°C on E16, E17, and E18).

Per treatment, 1,600 Ross 308 eggs from a 41wk old parent flock were set. On E18, eggs were transferred with 50 eggs per hatcher basket. At pull, the number of dead, first, and second grade chicks was determined per basket and a breakout was performed on unhatched eggs. Navel closure was scored from 1 (perfectly closed) to 4 (open, with a >2mm black button) in 5-7 baskets per treatment. Post hatch, 160 male chicks per treatment were transported and placed as groups of 20 in 8 replicate pens. BW and FI were determined per pen on d7, 14, 26, and 35. Mortality was recorded daily.

Hatch of first grade chicks from fertile eggs was higher for Control (+4.2%) than for Cold (P=0.005), with Spiking intermediate. Cold and Spiking resulted in 2.4 to 3.2% more second grade chicks than Control (P=0.002). Navel closure did not differ between treatments (P=0.24). ADFI from d0-d7 was 3.1g/day higher for Cold than for Spiking, with Control intermediate (P=0.049). BW on d26 was higher for Control than for Cold (+38.9g) and Spiking (+49.2g; P=0.03). This difference was created mostly by a higher ADG between d14 and d26 for Control than for Cold and Spiking (P=0.008). No differences between treatments were found on d35 (P>0.12). Total mortality did not differ between treatments (P=0.60).

To conclude, Control showed better incubation results and the hatched chicks performed better post hatch than Cold and Spiking up to d26, suggesting that an EST of 36.7°C from E18 onward was not beneficial.

Key words: Incubation temperature; Eggshell temperature; Post hatch performance

Transgenerational analysis of embryonic heat exposure in Japanese quail

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Changes in gene activity induced by perinatal environmental challenges are known to impact the phenotype, health and disease risk of animals. The epigenome is an essential contributor to phenotypic plasticity, and learning how environmental exposures translate into persisting epigenetic changes may open new doors to improve the robustness and resilience of developing animals. In that respect, birds are species of choice to directly manipulate the embryonic environment with limited direct maternal influence. It was previously reported that the heat tolerance of male commercial chickens was improved by cyclically elevating the egg incubation temperature. This procedure named embryonic thermal manipulation (TM) was associated an enhanced gene response when animals were heat challenged at slaughter age, 35 days post-hatch (D35). Unpublished work of our team shows that TM is associated with two epigenetic marks changes in the hypothalamus of D35 chickens that may contribute to the molecular basis of TM-induced programming of gene expression.

To further explore the molecular mechanisms of heat acclimation, we took advantage of an inbred line of Japanese quails (Coturnix japonica) to investigate the transgenerational impact of TM on bird epigenome. Among other advantages, the quail short generation cycle is 3-4 time faster than the one of chicken and the use of an inbred genotype should help reducing phenotypic variations due to genetic variability. TM was transposed to quail by elevating the incubation temperature from 37.8°C to 39.5°C during 12 hours per day from the 12th hour of incubation until E13 (E0-13). TM affected the hatching rate and the survival during the first four weeks of life, the growth until 25 days of age and the surface temperature of the shank at D35. We also found that TM impacted some blood metabolites in interaction with sex at D35. The thermal response of TM animals was assessed by a heat challenge at D35 that had no impact on survival. Nevertheless, according to beak surface temperature and blood sodium levels, TM animals differentially responded to the heat challenge, in in a sex dependant manner. To explore the molecular impacts of TM, a genome-wide study of gene expression by RNA-seq and of DNA methylation by whole genome bisulfite sequencing (WGBS) is currently ongoing on D35 hypothalamic tissues of TM and control animals.

TM was repeated on the progeny of TM animals to a total of 4 consecutive generations in order to evaluate the multigenerational impact of the treatment, in parallel to 4 generation of untreated animals crossed in a mirror manner as controls. In addition, we derived 2 generations of control treatments from two consecutive generations of TM to assess the transgenerational impact of TM. A phenotypic characterisation including physiological, reproductive and behavioural measurements is currently underway at all generations and the first results will be presented.

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Key words: Embryonic environment; Thermal Manipulation, Japanese quail; Transgenerational, Epigenetics.

Variations in incubation and postnatal environments affect the microbiota composition of fast-growing male chickens

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The caecal microbial population is known to influence the health and growth performance of the host, even when modified as early as at the embryonic step using in ovo inoculations. For favouring the developing chick embryo adaptation to thermal variations of later breeding environment, programs of temperature fluctuations during incubation were studied, such as those proposed for improving heat tolerance. The present study aimed to determine whether bacterial caeca composition was modified or not by variations in both incubation and postnatal environments in fast-growing male chickens.
Caecal bacterial communities were identified using high-throughput 16S rRNA gene sequencing techniques in 41 d-old chickens incubated either in control conditions (I0, 37.8°C and 56% relative humidity RH) or with temperature variations during incubation (I1; 39.5°C and 65% RH 12 h/d from day 7 to 16 of embryogenesis followed by 2 times 30 minutes at 15°C and 75% RH at days 18 and 19 of embryogenesis by transfer in a cold room). These incubation conditions were combined in a factorial design with control postnatal rearing conditions (T0 room with 33°C at d0 and temperature decrease down to 21°C until 3 weeks of age, which was maintained until 41 d of age) or challenging conditions in the T1 room with 28°C at d0, when chicks are sensitive to cold, a temperature decrease until reaching 21°C at 3 weeks of age, followed by heat exposure at 32°C from d 27 to 41, when chickens are sensitive to heat.
Results showed that the caecal microbiota composition of chickens from the T1 postnatal room differed strongly from that of chickens reared in the control T0 room. The experimental set-up cannot discriminate between the impact of thermal treatment and that of the bacterial environment of the room.
Interestingly, I0 and I1 caecal microbiota composition could be separated by discriminant analysis of principal components using 14 operational taxonomic units in the Control T0 room. Such discrimination, which was not observed in the challenge room T1 when considered alone, suggests that the incubation environment (temperature within each room/incubator) may affect the chicken caecal microbiota composition in the long term.
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Key words: Gut microbiota; Incubation; Temperature variations; Chicken

Liver ontogeny in mule ducks: determination of the best embryonic thermal programming window

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Our team recently demonstrated that different rises in incubation temperature during the second half of embryogenesis, led to a better fattening of the liver after overfeeding in all treated groups (up to 15%) compared to control group. However, despite an increase in the yield of fatty liver, two distinct thermal manipulation conditions (with exactly the same cumulative rise in temperature) resulted in a decrease in hatchability and a slight deterioration in the quality of the final product. Based on these results we suggest that fatty liver production could be optimized by a more precise embryonic thermal manipulation, in particular by a better knowledge of the embryonic establishment of the different physiological functions of the liver. To this end, a kinetic study was designed to determine the level of expression of different genes involved in steatosis-related liver function throughout embryogenesis. The livers of 20 embryos were collected every four days from the 12th day of embryogenesis until four days after hatching, and a Fluidigm real time PCR analysis was performed. The complete results presented in a heat map led us to classify the 86 genes measured at 7 sampling points into 4 major pathways expressed differently all along embryogenesis.First, most of mRNAs involved in lipid metabolism are overexpressed after hatching (FAS, SCD-1, ACLY and ACOX1). Interestingly, genes implicated in carbohydrate metabolism (HK1, InsR, GAPDH, ACSS1) and liver development (HGF, IGF and FGFR2) were predominantly overexpressed from E12 to E20 or E24. Finally, regarding cellular stress, the gene expression seems quite stable throughout development except for some mRNA witch presented a peak of expression at hatching (IDH1, CYP2E1 and HSP90AA1). Thermal manipulation applied on a large half of the embryogenesis (from E12 to E27) resulted in an increase in the production of fatty liver in mule ducks but also a reduction in the hatching or the quality of the final product. In this study, we highlighted for the first time the ontogeny of the liver in mule ducks, which may be useful for optimizing metabolic programming induced by thermal manipulation. Indeed, since the cumulative elevation threshold has already been determined, reducing the duration of thermal manipulation could allow different applications in order to improve the metabolic programming.

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Key words: Ontogeny; Liver; Incubation; Genes

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