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Liver ontogeny in mule ducks: determining the 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 the 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 optimised 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 seven sampling points into four major pathways expressed differently all along embryogenesis.

First, most 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 which 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 optimising 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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Variations in incubation and postnatal environments affect the microbiota

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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, programmes of temperature fluctuations during incubation were studied, such as those proposed for improving heat tolerance.

The present study aimed to determine whether the 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 day-old chicks 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 7-16 of embryogenesis followed by 2 x 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 day 0 and temperature decrease down to 21°C until three weeks of age, which was maintained until 41 days of age) or challenging conditions in the T1 room with 28°C at day 0, when chicks are sensitive to cold, a temperature decrease until reaching 21°C at three weeks of age, followed by heat exposure at 32°C from day 27-41, 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 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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Influence of chronic and short term perinatal temperature manipulations

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In precocial birds the hatching phase is a critical period in which perinatal temperature manipulation (PTM) induces long-lasting changes in physiological, peripheral and central nervous mechanisms. This review focuses on our own research projects on the 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 characterised 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 characterised by high warm and low cold sensitivity (D10 15% warm and 14% cold sensitivity).

PTM related modifications also show 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 players 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. ■

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Effect of feed and water deprivation during transport on early fed chicks

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Many chicks have immediate access to feed and water in the hatcher nowadays, while they are deprived of 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 six replicate pens containing 20 male chicks. Bodyweight (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 (Δ=74g; $P=0.02$), while no main effect was found for 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 (Δ=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). ■

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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, rearing with or without a broody hen, rearing with or without a dark brooder, and effects of genetic background on early life development. We also examined effects of housing single- or mixed-sex groups in broilers using these same tests and techniques. We showed that overcrowding during rearing elevates feather cortisol and increases anxious behaviour, 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 behaviours while not affecting the HPA axis.

Together these results emphasise 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. ■

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

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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 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 successfully 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 been developed and is likely to be more widely used in the future. Modern hatchery equipment continuously monitors the incubation environment and collects the data.

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 the hatchery as it is in all other aspects of business. ■

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Effect of female age and genotype on eggshell quality in ostrich

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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 breeding 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 two-years-old compared to females of three 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 2cm^2 than 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. ■

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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 and their impacts on the phenotype of their mule ducklings, obtained from an inter-generic crossbreeding with Muscovy drakes. A total of 60 female laying ducks were divided into two 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 10-51 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. ■

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