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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.

This presentation delves into the concept of transgenerational epigenetic inheritance in animals, providing key examples for many species. Special focus is placed on chickens and the impact of early stressors on their behaviour.

Advantages and drawbacks of studying transgenerational epigenetic inheritance in birds are underlined and future directions explored. ■

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Effect of egg weight loss and hatch time on chick yield

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This study investigated the effects of egg weight loss (EWL) and hatch time (HT) on chick yield and seven day post-hatch performance. Broiler hatching eggs were obtained from a commercial flock of Ross 308 at 40 weeks. Eggs (64g±2g) 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 weighed at 510h (take-off) of incubation to determine the chick yield (CY). The hatching process was divided into three time periods: Early (475-487 h), Middle (491-493 h), and Late (498-509 h). A total of 810 chicks were raised up to day 7 of age when Body Weight (BW), Feed Consumption (FC) and Mortality (M) were recorded. Data were analysed using a factorial random design of 3 EWL x 3 HT treatments.

Average 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 (44.3g) compared to Standard (43.5g) and High (42.2g) at 510 h ($P<0.05$) but this advantage disappeared by day 7 and there was no significant difference in BW and FC among EWL groups at day 7.

Late HT exhibited the greatest BW at 510 and 518 h, and the lowest BW and FC at day 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 day 7 ($P<0.05$).

The result of this study demonstrated that either EWL or HT affects CY, with only HT having a seven day long effect on performance. ■

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Transport of 18-day-old hatching eggs: effects on physiology and behaviour

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Incubation, hatching, and raising of broiler chickens 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 embryos 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, for example show a longer duration of TI, and higher levels of feather corticosterone compared to chicks that experienced short transport. ■

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Incubation temperature affects bursa of Fabricius histology of broiler chicks

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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 the most optimal organ development.

However, based on recent studies, it can be hypothesised 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 Ross 308 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 the incubators were opened every 6 h 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 haematoxylin 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 38.9°C resulted in a lower (p=0.02) cell density within follicles (39.2px) compared to an EST of 37.8°C (46.0px). Additionally, a lower EST in wk 3 of 36.7°C resulted in a higher (p=0.01) cell density within follicles (46.8px) compared to an EST of 37.8°C (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. ■

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Nutrient utilisation 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 utilisation 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 utilisation 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 decreased during the last 60 years and external losses have 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, in general, there seems to be a slight increase in efficiency. These results indicate that modern chicken strains are able to utilise 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 utilised by the avian embryo.

These changes are probably not only caused by genetic selection, but also by changes in incubation conditions and management. ■

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Chicken embryos: from eggshell decalcification to skeleton mineralisation

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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 five of incubation onwards.

However, the molecular actors associated with the function of this extra-embryonic membrane are still poorly characterised.

The objective of this study is to decipher the role of the CAM in the mobilisation of eggshell minerals and in subsequent mineralisation of the embryonic skeleton.

Sixty eggs (64.1±1.8g) from Rhode 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 analysing 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 mineralisation. 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 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 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 hypothesise that the kinetics of bone mineralisation 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 Rhode Island laying hens. ■

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