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Relative humidity, egg weight loss and hatchability

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Two experiments were conducted to determine the effects of different humidity levels during the first 19 days of incubation on egg weight loss (EWL), embryonic mortality, and hatchability. Broiler hatching eggs were obtained from commercial flocks of Ross 308 at 43 weeks in both experiments.

Eggs (66g±2g) were numbered and weighed individually before set and during transfer (day 19) to determine the egg weight loss. In addition, the specific gravity of eggs (SG) as an indicator of egg quality was calculated by Archimedes principle in Experiment two. SG were classified as 'Low' (<1.070g/cm^3), 'Medium' (1.070-1.085g/cm^3), and 'High' (>1.085g/cm^3). In all cases, the eggs were stored for two days at 18°C and 75% RH before set.

Hatching eggs were randomly assigned to three identical commercial incubators (Vision, Petersime) either Low (LH), Standard (SH) or High (HH) relative humidity from set to 19 days of incubation for both experiments. To eliminate the effect of EST, eggs were incubated at an EST of 37.8°C in this study.

Embryonic mortality and hatchability data were analysed using the Chi-square test. EWL was 12.9%, 10.9%, and 9.1% in LH, SH and HH groups respectively, in experiment one and in experiment two, EWL of LH, SH and HH groups were 12.7%, 11.1% and 8.8% respectively. In both experiments, EWL was affected by relative humidity ($P<0.05$), but relative humidity of incubator did not affect hatchability ($P>0.05$).

However, in experiment two, the eggs from Low SG group had a lower fertile hatchability (90.7%) compared to Medium SG (93.2%) ($P<0.05$) with High SG (91.9%) intermediate.

The results of this study demonstrated that the RH affects the EWL but does not affect the hatchability and SG is a better tool than EWL for predicting the exact hatch results. ■

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Egg turning in Western Capercaillie

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Parental behaviour and embryo development evolve together to provide the best nesting success. During incubation birds control conditions in the nest: temperature, humidity, carbon dioxide concentration and egg position. We know that behaviour is flexible and may be affected by weather conditions, predators presence, female condition and her nesting experience, as well as the stage of embryo development. The embryo may even communicate with parents when still staying in the egg. And so, observing the bird's behaviour during incubation we can learn how to improve incubation in incubators. Egg turning is one example, especially when artificial incubation in incubators is not as effective as when eggs are incubated by parents. Problems in captive breeding of the Western Capercaillie, which is related to high embryo mortality during incubation, is one of those cases.

In this study we assumed that egg turning is one aspect of incubation that may give a hint about causes of low hatching success of artificially incubated eggs. Observations were performed on 13 females kept in the Capercaillie Breeding Centre in Wisła Forestry during the entire incubation period and in three subsequent seasons. Digital cameras placed near nests were used in order to not disturb females by human presence. Birds were accustomed to recording equipment and did not react to its presence and sound. Results showed that the egg turning rate depended on incubation day ($F_{27,222} = 2.29$; $P = 0.001$).

At the beginning of incubation the egg turning was more frequent, especially on the first day. Frequency decreased to 8th day and up to 24th day stays at a similar level. When hatching begins, on 25th day, the egg turning frequency increased. The turning rate was affected by time of day ($F_{23,4437} = 12.15$; $P < 0.001$).

At night, between 20.00 and 03.00 females were sleeping, so they turned eggs sporadically at 0.76 turns per hour, while during the rest of the day it was 1.19 turns per hour.

It was interesting that the level of egg turning activity was highly characteristic of individual females ($F_{12,237} = 3.13$; $P < 0.001$), and did not differ between years ($F_{12,447} = 0.17$; $P = 0.840$).

Egg turning seems to be the most important in the first days of incubation. Our observations led us to speculate that during artificial incubation the egg should be turned until the hatching starts as egg turning may help the chick to find the best position. We believe that more observations on incubation behaviour should be done. ■

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Flock age, frequency of turning and SPIDES

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This study investigated the effect of broiler breeder flock age, frequency of turning, and SPIDES during 14d storage on the developmental stage of embryos and hatchability.

Hatching eggs were obtained from two Ross female line grandparent flocks from younger (29 wk) and, older (58 wk) ages and eggs were stored for 14 days at 15°C and 75% RH.

During storage, eggs were either held continuously in the storage room (Control) or were subjected to a heat treatment regimen delivering 3.5 hours above 32°C, in a Petersime Re-Store machine at day five of storage and turned 0 or 4 times daily during storage.

In each treatment, 15 eggs were opened in both flock ages at 5d of storage to examine the stage of embryonic development.

All eggs were set in a single incubator and hatcher. A tray of 150 eggs constituted a replicate and six replicate trays (900 eggs) were set per heating treatment at each turning frequency and flock age.

Embryonic development was advanced by SPIDES, turning frequency of four times and in eggs laid by the older flock.

Hatchability was significantly better for the younger flock compared to the older flock. Hatchability was improved by turning eggs four times daily compared to no turning during storage due to lower late embryonic mortality ($P<0.05$).

This effect was more evident for older flocks. SPIDES increased hatchability and reduced embryonic mortality and second grade chicks compared to the control in both flock ages ($P<0.05$).

It can be concluded that the highest hatchability was observed in eggs both turned four times and given one SPIDES treatment at five days of 14 day storage in both flock ages. ■

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Multigenerational effects of heat manipulation

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Fast-growing broilers are heat-sensitive animals, especially at slaughter age. Thermal manipulation during embryogenesis consisting of increasing incubation temperature of eggs up to 39.5°C and relative humidity (RH) to 65% for 12 hours/day between days 7 and 16 of incubation has been shown to improve their thermotolerance.

Previous studies have demonstrated the effects of these thermal manipulations on growth, physiology and meat quality of broilers reared in semi-commercial conditions, with impacts on their metabolism, gene expression and potential epigenetic mechanisms in the long term. The present study explored the effects of this treatment during incubation on body temperature and growth in the two following generations.

Parental broiler chicken (F0; Cobb 500) were either submitted to a control incubation condition during embryogenesis (C; 37.8°C and 56% RH) or treated (T) with cyclic exposure at 39.5°C and 65% RH for 12h/d from d 7 to 16 of incubation, and the resulting chickens were raised as breeders. F1 progeny were incubated under control conditions.

The resulting CC and TC chickens, respectively, were raised under standard breeder conditions and were reproduced taking into account parental origins. The resulting F2 eggs were incubated in control conditions to obtain CCC and TCC animals, raised as broiler chickens under standard conditions up to 41 days of age. Results showed no effect of incubation conditions on hatchability within each generation.

In F2, there was no effect of the initial incubation treatment on body temperature at hatching, unlike in F0.

However, at five days of age, body temperature was lower in TCC than in CCC male chickens. Growth was also altered, as demonstrated by the 8% higher body weight observed in TCC compared with CCC chickens at slaughter age.

Altogether these results provide the first line of evidence of a multigenerational effect of heat stimulation during embryogenesis on growth and thermoregulation in fast-growing chickens. ■

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Hatching egg quality – with a focus on the cuticle

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Eggshell quality has important consequences for hatching success and chick quality. It is also important for ensuring good hygiene in the hatchery. The eggshell provides both physical and chemical protection to the embryo and at the same time it must regulate the exchange of metabolic gases and water. The eggshell also serves as a source of calcium for the embryo.

These multifaceted requirements require a complex architecture. The structure of the shell was first described in the early 19th century. Since then biologists and engineers have used technologies such as scanning electron microscopy, x-ray diffraction, mechanical modelling and testing, and molecular methods to study the eggshell and how it is formed. Such investigations have provided new insights into the process of mineralisation, and how the different components (organic and inorganic) contribute to the eggshell's physical and functional properties.

Eggs are not laid in a sterile environment and so at the time of oviposition surface contamination of the eggshell is inevitable: the dirtier the surface the egg is laid onto, the higher the risk of contamination.

For the embryo, protection begins with the cuticle, a unique proteinaceous layer which covers the outside surface of the shell and plugs the gaseous exchange pores.

The cuticle is formed in the last 1.5 hours before oviposition and consists of a number of proteins, some with known antimicrobial activity. In birds that lay eggs in challenging environments, the cuticle is often thicker, suggesting evolutionary pressure for the trait. In the poultry industry, where there is reliance on artificial incubation of eggs to prevent the transfer of micro-organisms from one generation to the next, there has been no artificial selection for this trait.

Thus, the cuticle is often reported to be patchy or absent on hatching eggs. According to EFSA, vertical transmission from broiler and layer breeders to production flocks is still the most likely route of transfer of antibiotic resistant E. coli and salmonella.

Horizontal transmission can also occur during the collection and transport of eggs.

Given that a) cuticle deposition is known to be genetically determined in genetically divergent breeds of chicken; b) a good cuticle is known to reduce an egg's susceptibility to penetration by E. coli and salmonella; c) cuticle deposition appears to be protected over other egg quality traits from a bird age-related decline, improving cuticle deposition on eggs seems like a worthwhile breeding goal. Cuticle deposition can now be measured rapidly using the custom built LI200 Ecutimeter but a staining step is required.

Staining does not compromise embryonic development, so stained eggs can be incubated. Within the natural variation in cuticle deposition found in eggs from pure-line egg and meat types of chicken, no relationship with water vapour conductance has been observed.

Incorporating this measurement into breeding programs will therefore contribute to improving the biosecurity of eggs, by reducing vertical and horizontal transmission of potentially zoonotic and pathogenic organisms from parent to offspring, without any unintended consequences for the hatching egg. ■

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Nesting behaviour during egg laying period

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In birds two ways of onset of egg incubation are distinguishable. In case of asynchronous hatching, parents usually start incubation after the first egg is laid. Consequently, in a single nest the chicks are at different ages and very often the youngest one does not survive.

This pattern is found in altricial species, like Passeriformes and birds of prey. Opposite to this, the second strategy of synchronous hatching is typical for precocials like Galliformes and Anseriformes. In this case, parents, usually females, start incubation after the last egg is laid, and as a consequence all offspring hatch synchronously.

Shortly after that, chicks can follow parents. Before incubation starts, already laid eggs and embryos at early stage of development are exposed to changing conditions of the external environment. During three years of observations using digital cameras placed near the nests we followed the behaviour of 14 females throughout their laying period.

All observations were conducted in the Capercaillie Breeding Centre in Wisła Forestry where birds are kept in conditions similar to those in the wild, therefore eggs are exposed to changing weather conditions. Despite this, hatchability from fertilised eggs is around 80-100%.

We found that females lay an egg every second day, and that on average they spend 157 minutes in the nest (the shortest stay: 62 minutes, the longest 520 minutes). Clutches contain 5-10 eggs, usually 6-8. Eggs were laid mainly in the morning: between 4:00-8:00 – 50.65%; between 8:01-12:00 – 32.46%. Only 16.88% were laid between 12:01 and 16:00.

Beside visits related to egg laying, females also visited the nest for short periods of time, just to sit on the nest and warm the eggs (24 minutes on average). The frequency of these visits however was difficult to determine for the individual birds because females often entered the nests of other individuals, including non-monitored nests. We want to extend our research by observing more nests in future years.

Egg covering with nesting material, that may prevent them from being predated and protect against unfavourable changes in environmental conditions, was another observation. Females were more willing to cover the nest after an egg was laid ($F_{1,81} = 7.25$; $P = 0.009$). The length of time spent in the nest and the time of the day were not affected by egg covering behaviour.

Observing behaviour during egg laying in Capercaillie may suggest ways to store eggs and prevent early developed embryos from dying despite unfavourable external conditions. The first results are promising, but more observations are still required. ■

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Laying performance and egg quality

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The selection of meat-type lines for increased growth and muscle development has been accompanied by significant physiological changes. While considerable gains have been made in production performances, there are limits in terms of product quality but also reproduction.

There is, therefore, a real challenge to better understand and exploit, the elements of the compromise between production, reproduction and meat quality.

The study focused on breeder hens at the 11th generation of selection, at which a differential of 0.5 pH unit is observed for the selection criterion (ultimate pH measured in chicken growing in the breast muscle at six weeks).

Eighty females for the pHu+ and for the pHu- lines were housed in single cages in a controlled environment from 20-40 weeks of age. During this period, eggs were weighed every week and eggshell mechanical properties (eggshell shape, percent, index, thickness, toughness, elasticity) have been determined to evaluate the egg quality for each line during all the laying period. Data collected between 23 and 39 weeks of age show a decrease in the laying rate, a first egg delayed and a higher percentage of broken eggs in the high pH line (pHu+, line presenting the lower energy status) by comparison to the low pH line (pHu-). Divergent selection also changed the characteristics of the eggs, resulting in heavier eggs and a higher shape index value (more round shape eggs) in the pHu+ line.

In conclusion, these results suggest a deterioration in the reproductive performance studied and changes in the characteristics of eggs in relation to the decrease in energy storage caused by selection for an increase in pHu. These first observations pave the way for future genetic studies to evaluate the contribution of energy status in terms of improving reproductive traits whose degradation penalises the meat-type sectors. In an original way, they also suggest the possibility of identifying new indicators or biomarkers of the energy status from measures related to reproduction and the egg. ■

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Effect of SPIDES duration on embryonic development

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This study investigated the effect of broiler breeder flock age and SPIDES duration for 14 days storage on the developmental stage of embryos and hatchability. Hatching eggs were obtained from two Ross female line grandparent flocks at prime (37 weeks) and older (54 weeks) ages and eggs were stored for 14 days at 15°C and 75% RH.

During storage, eggs were either subjected to a heat treatment regimen delivering 3.5 or 5.5 hours above 32°C, in a Petersime Re-Store machine at day 5 of storage. In each treatment, 15 eggs were opened in both flock ages at 5d of storage to examine the stage of embryonic development.

All eggs were set in a single incubator and hatcher. A tray of 150 eggs constituted a replicate and seven replicate trays (1,050 eggs) were set per heating treatment at each flock age. Embryonic development was more advanced by longer SPIDES treatment in eggs laid by both ages.

As expected, prime flock showed significantly better hatch of fertile eggs (HOF) than older flock. HOF was significantly better for 5.5h SPIDES treatment duration in older flock ($P<0.05$) and numerically better in prime flock eggs when compared to 3.5 hours SPIDES treatment.

It can be concluded that the better hatchability was observed in eggs given 5.5h SPIDES treatment compare to 3.5h treatment at 5 days of 14d storage in both flock ages. ■

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Epigenetics and incubation: implications for scientists

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Developmental plasticity, foetal programming and epigenetics are all closely interrelated phenomena, but are often treated as separate entities or, worse, are ignored by animal physiologists and poultry scientists, alike. Yet, understanding how this triad can affect reproduction and survival, as well as create unwanted variation in our experimental data and commercial efforts, can lead to greater understanding of how environment influences processes such as avian incubation.

Environmental influences (for example incubation humidity as well as low oxygen, high temperature, and even hydrocarbon pollutants) affect ontogeny during the critical windows for developing birds, and potentially these effects may be epigenetically inherited and so last generations beyond the initial exposure.

While the focus is on avian incubation, several vertebrate animal models are used to probe developmental plasticity, foetal programming and epigenetics and their underlying molecular, morphological and physiological mechanisms.

While there are deleterious effects of exposure to stressors, there are also surprising adaptations allowing subsequent impacted generations to not only survive, but to thrive in the face of parental exposure to stressors.

Ultimately, short-term modifications in phenotype lasting at most a few generations may be a mechanism more effective than evolutionary, gene-modification based change for surviving shorter term, stochastic environmental stressors. These findings may translate into practical guidance for the poultry professional. ■

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Changes in DNA methylation in songbird brain

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The zebra finch is a songbird species commonly used as a model organism in neuroscience and behavioural ecology. Our past research has shown that vocal signals trigger changes in gene expression in cognitive centres of the adult zebra finch brain.

Meanwhile, new research suggests that zebra finch parents may influence the future metabolism of their offspring, through vocal signals emitted during gestation (Mariette & Buchanan, Science 2016). Ultimately, we plan to test the hypothesis that vocal signals to the egg trigger epigenetic mechanisms that support improved thermal tolerance in later life. As a foundation for this plan, we describe our recent analysis of DNA methylation in adult zebra finch brain. We used Reduced Representation Bisulfite Sequencing to identify changes in DNA methylation in the auditory forebrain of zebra finches after two days of isolation in a sound attenuation chamber, compared to group-housed controls.

We detected changes in methylation of several sites associated with the BDNF gene, including increased methylation at one of several promoters regulating alternative transcription start sites, and decreased methylation within the protein-coding domain of the BDNF gene.

These changes were accompanied by a down-regulation of BDNF mRNA. These results confirm that relatively brief environmental signals can trigger epigenetic changes which may be related to growth and adaptation and can be detected using high-throughput DNA sequencing methodologies. ■

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For details of the 2019 Incubation & Fertility Research Group Meeting visit www.ifrg.org or contact Dr Marleen Boerjan ifrg@pasreform.com