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The effect of various eggshell temperature patterns on hatchability

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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.

Three EST patterns were applied: Control (constant 37.8°C), Cold (37.8°C until 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 41 week 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 eight 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. ■

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Evidence for epigenetic reprogramming in response to acoustic signals

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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.

These adaptations include altered growth rates and thermal sensitivities – traits of interest to poultry producers.

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. ■

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Effect of chick holding temperature and provision of a hatchling supplement

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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 three 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 (six replicates of 45 chicks per treatment) and were grown to seven 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. ■

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Involvement of two-pore calcium channels in the regulation of cardiac activity

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Calcium homeostasis influences the cardiovascular function in the developing chick embryo. It is known 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 signalling.

We focused on the role of the two-pore calcium channels in the spontaneous rhythmic contractions of the chick embryonic heart.

These channels are localised 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 analysed 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 10μM) caused an inhibitory effect, and the HR decreased by 30%. When the trans-NED19 concentration was increased by two times (20μM) the HR decreased by about 42% of the value in the control and it did not significantly differ from the effect of 10μM. 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 10μM and 20μM 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 mobilisation of calcium from endolysosomes through the two-pore calcium channels activated by NAADP. ■

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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 with 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 to investigate the transgenerational impact of TM on bird epigenome. Among other advantages, the quail short generation cycle is 3-4 times 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 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 bisulphite 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 four consecutive generations in order to evaluate the multigenerational impact of the treatment, in parallel to four generations of untreated animals crossed in a mirror manner as controls.

In addition, we derived two 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. ■

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Long-lasting metabolomic faecal 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 a negative effect on their performance. Based on this observation, the metabolites from the faeces 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 faecal 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 faecal 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. ■

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Metabolomic analysis of nutrient sources in the embryonic egg

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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 chickens.

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, the embryo will use other nutrients present in the egg white and amniotic fluid. We hypothesised 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 analysed 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 characterisation 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. ■

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Heat treatments during prolonged storage of eggs from breeders

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Long periods of egg storage between laying and incubation cumulated with low qualitative flocks' ages degrades egg quality. These parameters are inherent to breeders' availabilities and market demand, and cannot always be optimised by hatcheries. This project aims to test different factors during egg storage to compensate for these critical but imponderable situations.

In order to improve broiler health and welfare and limit 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 Young breeder flock (28 weeks of age) and one Old 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 6 and 10 days post-lay – each lasting four 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 first 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 four.

Feed intake was not different between groups.

Heat treatments for young and old breeder 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. ■

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Effect of egg cooling on embryonic development 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 weeks old were used in Experiment 1 and 2, respectively.

A total of 3,180 eggs that had been laid within a 15 minute 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 eight hours at 27°C then sent to egg storage room. EST was cooled down to 24°C in three hours and 10 hours 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 four hours above 32°C, in a Petersime Re-Store machine at d4 of storage (SPIDES). Some (30 embryos in each batch) of the eggs were opened after cooling process (d0) and SPIDES (d4) to determine the stage of the blastoderm. Each tray of 150 eggs was considered to be a replicate and there were five replicate trays per heat treatment in each cooling profile treatment. The eggs were randomly set in a single commercial incubator.

Data from the two (cooling profile) x 2 (heat treatment) completely randomised 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 beneficially by SPIDES, in the case of the old flocks. ■

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