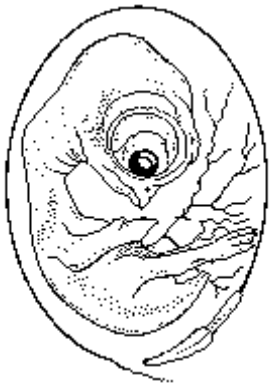

Incubation and Fertility Research Group
(IFRG/WPSA Working Group 6)

2018 Meeting – Edinburgh, Scotland

October 4th – October 5th

ABSTRACTS

Abstracts of presentations are in the order as scheduled in programme timetable; poster abstracts are at the end of the document. In author list * designates the presenter.

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Thursday, October 4th

10:30-11:30	**Registration**
11:45-12:30	**Welcome and lunch buffet**
12:30-12:45	**Marleen Boerjan and Dinah Nicholson** Introduction to programme

Session 1: Embryo quality, egg handling and egg storage

12:45-13:30	**Anke Förster***, Lohmann Tierzucht GmbH, Germany* ***In** ovo* sex determination: where are we now?
13:30-14:10	**Murray Bakst PhD,** *consultant, USA* Let's talk about isolating and staging embryos
14:10-14:30	**Ewa Łukaszewicz,** *University of Wroclaw, Poland* Stage of goose embryo development at oviposition depending on genotype, age and laying period
14:30-14:50	**Ampai Nangsuay,** *Aviagen, Thailand* Effects of egg handling in tropical climate SPIDES on embryo stages
14:50-15:00	**Plenary question and discussion**
15:00-15:30	**Coffee/tea break**
15:30-15:50	**Joanna Rosenberger**, *University of Wroclaw, Poland* Egg turning in Capercaillie *(Tetrao urogallus)*
15:50-16:10	**Okan Elibol,** *Ankara University, Turkey* The effect of rapid cooling rate of broiler hatching eggs after oviposition on embryonic development and hatchability
16:10-16:30	**Tolga Erkus,** *Aviagen, UK* Effect of SPIDES duration and flock age on embryonic development and hatchability of long-stored grandparent eggs
16:30-16:45	**Plenary question and discussion**
16:45-17:45	**POSTER AND DEMONSTRATION SESSION**
16:45-17:45	**Practical demonstration session** "Staging the Embryo" **Murray R Bakst** *(consultant addressing issues in fertility and early embryo mortality)*

19:00 CONFERENCE DINNER

Friday October 5th

Session 2: Egg quality

09:00-09:20	**Marleen Boerjan***, Pas Reform, The Netherlands* Differentiation of embryonic cells as a result of gastrulation-related movements in the blastoderm
09:20-09:40	**Roger Banwell***, Petersime nv, Belgium* Effect of specific gravity of hatching eggs and relative humidity on egg weight loss and hatchability
09:40-10:30	**Maureen Bain,** *University of Glasgow, UK* Hatching egg quality – with a focus on the cuticle
10:30-10:40	**Plenary questions and discussion**
10:40-10:50	**Anne Collin,** *INRA, France* Introduction to combined meeting 2019 in Tours
10:50-11:15	**Coffee Break**

Session 3: Epigenetics, incubation and chick vitality

11:15-12:15	**Warren Burggren,** *University of North Texas, USA* Epigenetics and incubation: short- and long-term implications for scientists and practitioners
12:15-12:30	**Plenary questions and discussion**
12:30-13:00	**Lunch Buffet**
13:00-13:20	**Juan Lopez,** *Hendrix Genetics, Canada* Improper incubation temperature can contribute to lower hatchability and compromise poult quality
13:20-13:40	**Serdar Őzlü,** *Faculty of Agriculture, Ankara University, Turkey* Effect of hatching time and post-hatch holding time on yolk sac weight and broiler live performance
13:40-14:00	**Keith Bramwell**, *Jamesway Incubator Company, Canada* Effect of early feed and water access on broiler performance and processing yield
14:00-14:20	**Roos Molenaar,** *Wageningen University Research, The Netherlands* Eggshell temperature pattern during incubation affect leg bone characteristics of broiler chickens at slaughter age
14:20-14:40	**Joanna Rosenberger**, *University of Wroclaw, Poland* Nesting behaviour during egg laying period in captive kept Capercaillie
14:40-15:00	**Jean De Oliveira,** *Cargill, Belgium* Assessment of food safety risk and microbiome links from breeders through hatchery to meat broilers under commercial conditions
15:00-15:20	**Amjad Farzinpour,** *University of Kurdistan, Iran* Use of aromatase inhibitor as an alternative for contraception in laying quails
15:20-15:40	Presentation offered by **Muntaser Salem**, *Institute Poultry Resources, Amman, Jordan*
15:40-16:00	**Coffee Break**
	Close and future meetings - **Marleen Boerjan**

Posters

Alessandro Franzoni, *University of Pisa, Italy*
Incubation traits and embryo development in two broiler breeder pure lines divergently selected on their energetic status

Ewa Łukaszewicz, *University of Wrocław, Poland*
Effect of some organic additives to semen extender on rooster sperm quality after 6 hours of storage

Anne Collin, *INRA, Université de Tours, France,*
Multigenerational effects of heat manipulation during embryogenesis on body temperature and growth in broiler chickens

Julia George, *Queen Mary University of London, UK*
Rapid experience-dependent changes in DNA methylation in songbird brain

Okan Elibol, *Ankara University, Turkey,*
Effect of flock age, frequency of turning and SPIDES during storage on embryonic development, and hatchability of long stored eggs

Session 1: Embryo quality, egg handling and egg storage

In ovo sex determination: where are we now?

Dr. Anke Förster*

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The negative correlation between body weight and reproductive traits has led to specialized breeds either for efficient egg production or high growth rate. As the male offspring of layer lines don't have the potential for efficient fattening performance, they are commonly culled directly after hatch. From an animal welfare point of view, this is hardly acceptable and so breeding companies and scientific groups aim at finding a practical solution.

Three major ways are discussed:

- Breeding of dual purpose lines
- Rearing and fattening of layer line males
- *In ovo* sex determination

With dual purpose birds compromises are made, and neither egg production nor growth rate can reach sufficient and efficient results. Males of layer lines require much more feed and time to gain weight than specialized broiler lines, and at slaughter the carcasses do not match the customers' needs concerning valuable parts and meat quality. Being not comparable to specialized breeds from an economical and ecological point of view, both approaches still have to be considered as "niche products" with special requirements of marketing.

As a third alternative different approaches to determine the sex of an embryo at an early stage of incubation have been under investigation for more than 10 years. Several prerequisites are necessary to establish a practical method for industrial use: it has to be fast, reliable/precise, with no detrimental effects on hatchability, health and production rate, and it must be carried out before onset of pain perception. In Germany mainly two strategies showed promising results in laboratory and field studies: hormonal analysis of allantoic fluid and Raman spectroscopy.

It has been shown that the allantoic fluid in female embryos on day 9 or 10 of incubation contains significantly higher levels of estrone sulphate than the male. Sexing accuracy was over 95% and the impact on hatchability, health and performance was low to negligible. At a significantly earlier stage of incubation (day 3-5) optical methods can be applied, as they use the genetic information of blood cells. Most of the optical methods require a perforation of the egg shell. For Raman spectroscopy a stimulation laser is focused on a blood vessel and the reflected scattered spectrum can be analysed for gender differences. Reliability of gender determination has shown to be >90%, whereas the impact on hatchability is a little higher (~5% loss) due to the hole in the shell.

In the last few years more and more possible solutions have been published and discussed, such as genetic engineering or Magnetic Resonance Imaging. As yet those approaches still have to prove their reliability, applicability and consumer acceptance, while Raman spectroscopy and hormonal analysis are now at a stage for automation and possible large scale use.

Let's talk about isolating and staging embryos

Murray R. Bakst*, PhD

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Staging entails the classification of the normal sequence of embryonic development from the first cleavages of the fertilized germinal disc to the day of hatch. Individual stages are based on the morphological characteristics of the embryo when examined and not the number of hours post-oviposition or hours or days incubated. Staging should be used when a precise, objective, and repeatable assessment of embryonic development is necessary. Hamburger and Hamilton (HH, 1951) published the most widely used 'normal table' for domestic fowl describing embryonic development from oviposition (Stage 1) to the day of hatch (Stage 45). However, embryonic development actually begins about 7 hr after fertilization and continues through oviposition. It was Eyal-Giladi and Kochav (EGK, 1975) that published a normal table describing embryonic development from the first cleavage division (Stage I) through oviposition (Stage X) into the onset of pre-primitive streak formation (Stage XIV) during the initial hours of incubation.

Staging procedures continue to be used by developmental biologists in describing the origin and development of the initial germ layers through the morphogenesis of the complete chick embryo. With the advent of molecular biology, biologists use staging to define stage-specific gene expression associated with morphogenetic events. In the past decade staging embryos has found a niche in the fine tuning of hatchery egg storage techniques prior to incubation. By subjecting broiler eggs to SPIDES (short periods of incubation during egg storage) the hatchability of stored eggs is significantly increased. This is most likely due to the embryo (the blastoderm) advancing from Stage X (EGK) to Stage XIII (EGK), thus rendering the blastoderm more resistant to the stress of egg storage. SPIDES treatments that push embryonic development into early primitive streak formation [Stage 2 (HH)] will increase embryo mortality upon incubation. Staging embryos is not a routine task but it is a quality control measure that should be done if the hatchability of eggs following SPIDES treatment is not reaching target goals.

Preparation of the blastoderm for staging involves two steps: first, the blastoderm and its associated yolk are isolated from the surface of the ovum (yolk) and transferred to a Petri dish; and second, the blastoderm is exposed by removing the yolk masking its ventral surface. The first step is the same procedure used to isolate the perivitelline layer (PL) overlying the blastoderm for PL sperm-hole counts (see references). However, unlike the PL sperm-hole assay, when staging the blastoderm must remain associated with the PL, thus the need for the second step. Failure to adequately remove all the yolk lining the ventral surface of the blastoderm will hinder one's ability to differentiate the subtle differences that characterize the stages between Stage X (EGK) and the onset of primitive streak formation [Stage 2 (HH)]. The presentation will review these steps with the insight of over 25 years of experiential experience in staging embryos.

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Stage of goose embryo development at oviposition depending on genotype, age and laying period

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Embryo development and hatched chick quality are influenced by parental genotype, age, nutrition, environmental conditions, and flock management. It was the aim of this study to determine if breeder genotype, age of breeder, or eggs laid near the onset of egg production versus eggs laid near the end of egg production influence the stage of embryo development at oviposition.

To compare genotypes (Exp. 1) 50 eggs of comparable sizes were collected from 3-year-old commercial line White Koluda® (WK) breeders and from two breeds involved in a genetic resources conservation program, Zatorska (Za) and Bilgoraj (Bi). Age comparison (Exp. 2) was conducted with 50 eggs of comparable sizes collected from 1-, 2-, 3-, and 4-year-old WK breeders. To compare laying periods (Exp. 3), 150 WK eggs were collected the first week of March and 100 WK eggs collected the last 2 weeks of June.

All eggs were stored for 72 hr at 16°C and then staged using Eyal-Giladi and Kochav (EGK, Roman numerals) and Hamburger and Hamilton (HH, Arabic numerals) procedures. Digital images of the embryos were taken using NIS Elements software and Nikon SMZ800T stereo microscope equipped with a Nikon DS-Fi1c camera.

Exp. 1 GENOTYPE: Individual breed differences were evident with Stage X embryos comprising 42.4%, 33.3%, and 38.7% in the eggs examined from the WK, Bi, and Za breeds, respectively. For all breeds combined, 38.8% of the embryos were in Stage X. but in the next order in WK there was stage XI (18.2%), while in geese from the genetic reserve it was stage XIII (Bi – 14.3; Za – 26.4%).

Exp. 2. AGE: In eggs from 1-, 2-, and 3-year-old WK breeders, the majority of embryos (38.7, 32.4 and 42.2%, respectively) were at Stage X. In contrast, the majority of embryos observed in the 4-year-old WK eggs were in Stage XI (36.1%).

Exp. 3. LAYING PERIOD: With WK eggs staged in March and in June, the highest percentage of embryos were in Stage X (33.7% and 43.6%, respectively). In addition, more developmentally advanced stages (XI-XIII) was similar in both periods. However, embryos developmentally at the onset of primitive streak formation (Stage 2 HH) were only observed in in the eggs from the end of the laying season. Interestingly, earlier stages of development (Stages VI-IX) were observed exclusively in the eggs collected in March (early egg production). Results obtained encourage us for further experiments on factors affecting the stage of goose embryo development at oviposition and its impact on gosling hatchability.

The experiments and participation in IFRG 2018 Symposium were supported partly by the Ministry of Science and Higher Education for statutory activity of Wroclaw University of Environmental and Life Sciences and by Wroclaw Centre of Biotechnology program "The Leading National Research Centre (KNOW) for years 2014-2018".

Effects of egg handling in tropical climate and SPIDES on embryo stages

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The longer the egg storage interval between lay and setting, the higher the rate of early embryonic mortality (EEM). Short periods of incubation during egg storage (SPIDES) will reduce EEM because the SPIDES advances the stage of embryonic development from Stage X (Eyal-Giladi and Kochav, 1976), the normal stage of development at oviposition, to around Stage XIII a targeted stage of embryo development. This slight advance renders the embryo more resistant to the stress of prolonged cool egg storage. In tropical climates temperature control is challenging within the hen house, egg storage areas, and during egg transport to a hatchery. Until eggs are sufficiently cooled (below 22°C) embryo development may advance beyond Stage XII or XIII, and SPIDES would actually increase EEM. In this study, embryo stages were determined using Grand Parent (GP) hatching eggs obtained from different field situations in a tropical climate in order to determine the range of embryo stages before and after egg cooling and SPIDES.

Total of 361 Indian River GP hatching eggs were assessed for embryo stage. They were sourced from 1st or 2nd collection in three farms (27, 39 or 63 weeks old) and assessed immediately after collection, on arrival at the hatchery and after SPIDES treatment. House, air and eggshell temperature (EST) during holding, transportation and storage (including during SPIDES treatment) were recorded for each group. Flock age did not show a clear influence on embryo stage in the fresh eggs. However, the prime flock was a long way from the hatchery, and eggs were held on farm for 31 h followed by 18 h in transit, with EST always above 22°C. Conversely, the old flock eggs were cooled to below 22°C within 5.30h. During storage and transport, embryos in the prime flock eggs advanced from Stage 10.3 in to Stage11.7, whereas embryos in eggs from the old flock did not show any further development (Stage10.5 at both points). After SPIDES treatment, the embryo stages of both prime and old flock eggs developed to more advanced stages, from Stage 11.7 to Stage 13.1 in prime flock eggs and Stage 10.5 to Stage 12.7 in old flock eggs.

In conclusion, local environmental conditions affected the rate of embryo development during farm storage and transport. Furthermore, SPIDES treatment advanced embryonic development in prime and old flock eggs.

Egg turning in Capercaillie (*Tetrao urogallus*)

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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 when eggs are incubated by parents. Problems in captive breeding of the Western Capercaillie (*Tetrao urogallus*), which is related to high embryo mortality during incubation, is one of those cases.

In this study we assumed that egg turning is one of incubation aspects 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 was 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 a 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 a 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.

This experiment was financially supported by the National Research Centre- grant No. 2016/21/B/NZ9/02084, while participation in the conference by Wroclaw Centre of Biotechnology, program "The Leading National Research Centre (KNOW) of Wroclaw University of Environmental and Life Sciences for the years 2014-2018".

The effect of rapid cooling rate of broiler hatching eggs after oviposition on embryonic development and hatchability

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This study investigated the effect of the egg cooling profile of broiler hatching eggs after oviposition on embryonic development and hatchability of fertile eggs. Hatching eggs were obtained from Ross 308 broiler breeders at 28 wk (young) and 64 wk (old) of age. A total of 3,150 eggs that had been laid within a 15 minute period were collected and then randomly assigned to two temperature controlled chambers with either control (360'- 480') or rapid (45'-120') cooling to 24 and 18°C EST, respectively. Eggs remained in the chambers until the EST of both cooling groups were similar, then eggs were transported to the hatchery and were stored for 6 days at 16°C and 75% RH. Each tray of 150 eggs was considered to be a replicate and there were 5 replicate trays per cooling profile treatment in each flock age. Some (25 embryos in each batch) of the eggs were opened before and after cooling profile treatment to determine the stage of the blastoderm. The eggs were randomly set in a single commercial incubator. Data from the completely randomized design were subjected to ANOVA using the GLM procedure of SAS.

The stage of embryonic development was advanced by control cooling and by the older flock. In younger flock eggs, fertile hatchability was significantly decreased by rapid cooling due to higher early and late embryonic mortality ($P \leq 0.05$). However, early embryonic mortality and percentage of second grade chicks was reduced ($P \leq 0.05$) and fertile hatchability was numerically higher by rapid cooling compare to control in older flock eggs. In conclusion, the data from this study demonstrated that rapid cooling after lay retarded the stage of blastoderm development in eggs from both young and old broiler breeder flocks. This was apparently detrimental, as indicated by higher early and late embryonic mortality, in the case of the young flock but beneficial in the case of the old flock. The hatchability differences between young and old flock eggs induced by a rapid cooling rate might depend on the differences of embryonic development at oviposition.

Keywords: hatching broiler egg, egg cooling rate, blastoderm development, hatchability

Effect of SPIDES duration and flock age on embryonic development and hatchability of long-stored grandparent eggs

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This study investigated the effect of broiler breeder flock age and SPIDES duration for 14d storage on the developmental stage of embryos and hatchability. Hatching eggs were obtained from two Ross female line grandparent flocks at prime (37 wk) and, older (54 wk) 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 7 replicate trays (1050 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 compare to 3.5h 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.

Keywords: hatching eggs, embryonic development, SPIDES, flock age, hatchability

Session 2: Egg quality

Differentiation of embryonic cells during first days of incubation as a result of gastrulation-related movements in the blastoderm

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By definition gastrulation is the formation of primitive differentiated tissues by overall movements of embryonic cells during first 24 hrs of incubation. A process that is required for optimal embryo formation in the hen. If we have a closer look at the chicken embryo after 24 hr of incubation we can see a difference from an unincubated embryo. Within 24 hr the flat disc located on top of the yolk of an unincubated egg has developed to an elongated bilaterally symmetrical structure: the embryo. In this 1-day old chick embryo we can easily recognize the primitive streak as the origin of the future backbone of the chick. The transition from a flat disc, the blastoderm, to an elongated one-day chicken embryo is the result of the gastrulation process initiated by the increasing incubation temperature. The higher embryo temperature initiates an enormous movement of the pluripotent blastomeres to form three differentiated cellular layers: the germ layers. The different germ layers received instructions while they migrate towards their final position and are no longer pluripotent. The outer germ layer, ectoderm, will develop to surface layers (skin) and brain and nervous systems. The endoderm received instructions to develop the digestive tract, associated organs and lungs. The layer of cells between the ectodermal and endodermal layer, the mesoderm, generates connective tissues, blood cells, heart, kidneys, bones and muscles. Also the non-reproductive tissues of the gonads is generated from the mesoderm, and the future reproductive cells, oocyte or spermatozoa, are set aside before the gastrulation starts. By definition: the totipotent fertilized oocyte can develop into all cells in the body, including reproductive cells (primordial germ cells) and extra-embryonic cells. The pluripotent cells can no longer develop to extra-embryonic tissues or primordial germ cells. The multipotent cells in the three germ layers are restricted to form specific cell types like lung, brain and blood cells. In summary gastrulation comprises the process of differentiation of the pluripotent blastomeres to the multipotent ectodermal, endodermal and mesodermal cells. Each of these germ layers can be recognized, not only by their position in the embryo but also by differences in proteins produced. The movement of the pluripotent blastomeres in the unincubated blastoderm initiated differential gene expression. The differential gene expression is the result of the gastrulation related cellular movements. The movement of the cells creates, for each cell, a different time dependent 'micro-environment' which results in the formation of three basic germ layers. Each of these layers is recognized by their specific form and function of the cells as a result of the production of cell-specific proteins. Gastrulation-related movement of the blastomeres starts with the increased temperature of the blastoderm. However the initiation of cellular movements is not random but starts at defined 'organizer' region in blastoderm differentiated during embryo development in the hen.

Recently it has been shown that the *in utero* development of the pluripotent cells in the blastoderm depends on integrated networks of differential gene expression and protein formation. These integrated networks are already initiated before fertilization while the yolk is deposited in the follicles of the ovary.

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Effect of specific gravity of hatching eggs and relative humidity on 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 d of incubation on egg weight loss (EWL), embryonic mortality, and hatchability. Broiler hatching eggs were obtained from commercial flocks of Ross 308 at 43 wk in both experiments. Eggs (66g±2g) were numbered and weighed individually before set and during transfer (d19) 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 2. SG were classified as "Low" (<1.070 g/cm^3), "Medium" (1.070-1.085 g/cm^3), and "High" (>1.085 g/cm^3). In all cases, the eggs were stored for 2 d 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 d 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 analyzed using the Chi-square test. EWL was 12.9%, 10.9%, and 9.1% in LH, SH and HH groups respectively, in experiment 1 and in experiment 2, 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 2, 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.

Keywords: humidity, specific gravity, egg weight loss, hatchability

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 eggshells 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 1.5h 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.

Session 3: Epigenetics, incubation and chick vitality

Epigenetics and incubation: short- and long-term implications for scientists and practitioners

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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. In this seminar, I describe how environmental influences (e.g. incubation humidity as well as low oxygen, high temperature, and even hydrocarbon pollutants) affect ontogeny during the critical windows for developing birds, and how potentially these effects may be epigenetically inherited and so last generations beyond the initial exposure. While the focus will be 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.

Improper incubation temperature can contribute to lower hatchability and compromise poult quality

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It has been reported that the temperature is a crucial factor affecting poultry embryo development**,** hatchability, quality and farm performance. The majority of the studies are made in *Gallus gallus domesticus* and are extrapolated to *Meleagris gallopavo* without previous verification. In incubation trials often environmental air temperature is used as treatment applied to the eggs**.** Therefore, the purpose of this study was to compare the effect of four different levels of embryo temperature 99.2-99.4°F; 99.4-99.6°F; 99.8-100.2°F; and 100.5-101.0°F (shell temperature) frequently observed in commercial hatcheries from day one to twenty five, upon hatchability, poult weight and seven days mortality.

Turkey eggs (2,400) from one breeder flock (41 wk of age) were collected, stored for 3 d at 60°F, prewarmed for 6 h to 70°F, selected for a uniform egg weight (large and small eggs were culled at setting), and set in trays at 75 eggs/tray. Eggs were divided into 4 incubators and incubation air temperature was adjusted daily to correspond to 4 different eggshell temperatures (EST) from 1 to 25 d of age: 99.2–99.4°F; 99.4–99.6°F; 99.8–100.2°F; and 100.5–101.0°F (all treatments incubated at 53 % RH and turned hourly). Each day, shell temperatures were taken from the same 10 eggs from the center of the treatment set. If adjustments were made, EST were checked an hour later for accuracy. After 25 d of incubation, eggs were candled and the developing embryos were transferred into one hatcher with hatch trays stacked in a randomized manner; non-developing eggs were broken and stage of embryonic development determined. At hatch (28 d and 4 h), a sample of poults (25/treatment; 5 poults randomly chosen/hatch tray) were euthanized and residual yolk sacs were weighed and yolk-free poult weight calculated. Hatch residue was evaluated to determine stage of development.

The total hatch of eggs showed that those incubated at an egg shell temperature (EST) of 99.2-99.4°F and 99.4-99.6°F had a higher hatchability than the eggs incubated at 100.5-101°F at P≤ 0.10 level. The hatch for fertile eggs incubated at the 99.2-99.4°F and 99.4-99.6°F EST were significantly higher than the 99.8-100.2°F and 100.5-101°F EST treatments at P≤ 0.05. It has been reported in broilers that a high embryo temperature (102.2°F), but not low embryonic temperature (98.06°F) produces a higher embryonic mortality**.** The treatment 99.2-99.4°F and 99.4-99.6°F generated poults heavier at pulling time than EST treatment 99.8-100.2°F and 100.5-101°F. The yolk free body mass of the poults under the temperature treatments of 99.2-99.4°F and 99.4-99.6°F was higher than 99.8-100.2°F, but only 99.2-99.4°F was higher than 100.5-101°F poults.

The lower body weights and yolk free body mass found for the high temperature treatment are in agreement with studies made in broilers. It is suggested that the higher temperature accelerates the embryo growth and development so reducing the time for nutrient use plus it causes a lower efficiency of protein utilization for growth**.**

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Effect of hatching time and post-hatch holding time on yolk sac weight and broiler live performance

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The objectives of the present study were to determine the effect of normal holding versus extended holding on yolk sac weight, BW gain, feed consumption, FCR, and mortality of early, middle, and late hatching chicks. Broiler hatching eggs were obtained from a commercial flock (Ross 308) and at 49 wk of age were used in this experiment. Hatching was divided into Early hatch of 471-474 h, Middle hatch of 483-486 h, and Late hatch of 493-496 h. Half of the chicks were pulled and placed as normally would be the case at 504 h of incubation to create a duration of holding from time of emergence from shell (EFS) of 30, 18, and 6 h for Early, Middle, and Late chicks, respectively, which was an average of 18 h. For comparison, half of Middle and Late chicks were also pulled and placed 30 h after their EFS under optimum conditions. BW and yolk sac weight were determined at EFS and placement time (PT). Individual BW and pen feed consumption were determined at 7, and 35 d of age. To avoid confounding, all chicks were weighed at the same age relative to placement on feed in order to evaluate the true effects of post-hatch holding and/or time of placement onto feed in the growing house. Mortality was recorded daily. Data were analyzed using a completely randomized design with 3 (hatch time) x 2 (holding time) factorial arrangement of treatments. There were no significant differences in BW, and relative yolk sac weight at EFS. Late hatched chicks exhibited a greater relative yolk sac weight than Early hatched chicks at PT ($P<0.05$) but there was no difference after 30 h holding in all groups. Initial BW of chicks was reduced by 30 h versus 18 h holding, as expected, but 7 d from the time of placement on feed, feed consumption and BW gain of 30 h held chicks was significant greater than that of chicks placed normally (18 h holding) from the same EFS groups in this experiment ($P<0.05$), but were similar at 35 d. Mortality did not differ among treatments. Contrary to other recent speculations, an increased post-hatch holding period for chicks under optimum conditions may not necessarily be detrimental because there appeared to be a certain amount of time required without feed after initial emergence from the shell before the chicks were ready to consume feed aggressively.

Keywords: broilers, hatching time, post-hatch holding time, yolk sac, BW, feed consumption

Effects of early feed and water access on broiler performance and processing yield

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Industry practices aim to start chicks on feed and water as soon as possible after hatch, which has led to methodological and equipment developments providing nutrients for chicks before placement. The objectives of this study were to investigate the effects of having feed and water available to chicks in hatch baskets. At transfer, eggs were randomly designated as a control (no nutrient provisions) or treatment (feed and water provided) and placed in a corresponding hatch basket within a lighted cabinet. Each basket was divided in half by a partition with eggs on one side. The treatment baskets had containers of feed and water in the corresponding hatch basket end without eggs. The hatch window was divided into four periods. At the end of each period, hatched chicks were individually weighed, tagged, and placed back in the same basket, but on the opposite side that was either empty (control) or had feed and water (treatment). At the designated hatch pull time, a total of 1,352 chicks were randomly distributed by treatment throughout pens in the same house. Feed and water were provided *ad libitum* and all other management specifications were set according to industry standards. Bird weight (by pen and period) was recorded at placement and weekly to six weeks. Feed consumption was measured per pen on the same schedule. Mortality, culls, and associated weights were recorded. Individual bird weights were taken at days 21 and 42. At placement and day three, yolk sac, ileum, and liver samples were collected for evaluation from one random chick in each pen and group. At 43 days of age males from each treatment period in each pen (366 total) were processed. There were no significant interactions between hatch period and treatment. Hatching period influenced ($P < 0.05$) body weights of birds throughout the trial. Chicks with hatcher feed and water access were one gram heavier at placement ($P < 0.0001$) and remained heavier through day 28; afterwards there was no significant difference in body weight as compared to the control group. No differences in feed conversion, mortality, or carcass data were observed. Results of this study suggests that access to feed and water prior to chick placement may impact the body weight of broilers during early growth, but has no influence on final body weight, processing yield, feed conversion, or liveability.

Keywords: broiler, nutrition, early, feeding, hatch

Eggshell temperature pattern during incubation affects leg bone characteristics of broiler chickens at slaughter age

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Leg problems leading to a poor walking ability or lameness can negatively affect broiler chicken welfare and performance. Controlling this issue may be possible by improving leg bone characteristics, using a multi-factorial approach throughout the complete poultry production chain. One of the predisposing factors may be incubation temperature (Oviedo-Rondón *et al*., 2009). Recent findings suggest that chicken development can be improved with a high eggshell temperature (EST) of 38.9ºC in the second week of incubation or a low EST of 36.7ºC in the third week of incubation (Maatjens *et al.*, 2016; Nangsuay *et al.*, 2015). However, effects of these EST or combinations of these EST on post-hatch performance and in particular leg health are unknown. The current study evaluated effects of different EST patterns during incubation on leg bone characteristics of male broiler chickens at slaughter age. Eggs of a Ross 308 broiler breeder flock (44 weeks) were incubated in a 2×2 factorial design with a control (37.8ºC) or high (38.9ºC) EST in the 2nd week of incubation and a control (37.8ºC) or low EST (36.7ºC) in the 3rd week of incubation.

Results showed that body weight and proximal length of the tibia at slaughter age were not affected by EST treatment (all P>0.10; n=128). Lateral cortex thickness (+3.1%) and proximal bone head thickness on the metatarsal side (+1.3%) was slightly higher in the high compared to the control EST in the 2nd week of incubation (both P=0.04). The tibial breaking strength was 5.8% higher for the high EST compared to the control EST in the 2nd week of incubation (P<0.001) and 3.7% lower for the low compared to the control EST in the 3rd week of incubation (P=0.02). An interaction was found for proximal bone head thickness on the femoral side; the combination of a high EST in the 2nd week of incubation with a control EST in the 3rd week of incubation gave the highest value (P=0.05). It can be concluded that EST during incubation can affect leg bone characteristics in broiler chickens at slaughter age. The slightly thicker bone combined with a higher bone strength as a result of a high EST in the 2nd week of incubation might be related to changes in bone ossification during incubation or higher post-hatch activity (Molenaar *et al.*, 2018). The lower bone strength after a low EST in the 3rd week of incubation might be related to a longer incubation duration (+5 hours), resulting in a longer period that the chicken may experience a relatively low body mineral status (Yair *et al.*, 2012).

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Nesting behaviour during egg laying period in captive kept Capercaillie

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In birds two ways of onset of egg incubation are distinguishable. In case of asynchronous hatching, parents start usually incubation after the first egg is laid. As a consequence 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 o 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 behaviour of fourteen 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 fertilized eggs is around 80-100%.

We found that females lay 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 to 10 eggs, usually 6-8. Eggs were laid mainly in morning hours: 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 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 of environmental conditions, was another observation we made. 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 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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Assessment of food safety risk and microbiome links from breeders through hatchery to meat broilers under commercial conditions

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Intestinal microbial colonization is an important development process that takes place in early life. Colonizing bacteria are believed to come mostly from the mother or the environment. Since current poultry production relies on artificial incubation, the contribution of the breeder hen microbiota to offspring intestinal microbiome has been questioned. This study was designed to investigate the link and contribution of breeder hen microbiota to broiler chicken flocks under commercial conditions. Twenty excreta samples were collected directly from the cloaca using sterile swabs from six Ross 308 breeder flocks ranging between 35 and 52 weeks of life. These flocks were fed the same feed and kept under similar management conditions. Meconium samples were collected from 20 chicks from the eggs produced by these same breeder flocks on day of hatch at the hatchery. Finally, cloaca swab samples were collected from 20 broilers from these same birds at 21 and 32 days of age. DNA was extracted from each of these samples and submitted to one step PCR amplification of 16S bacteria DNA while incorporating a fluorescent dye. The individual labelled DNA samples were hybridized on a microarray chip containing a predefined list of 100 bacteria selected to be related to broiler performance and food safety. The image of fluorescence signals were captured and submitted to quality control and data analysis. The patterns were identified by Principal Component Analysis (PCA) and the flocks were compared by ANOVA with flock (1-6) and category (breeder, hatchery, offspring 21d and offspring 32d) and their interaction as fixed effects. Means were compared by FDR 0.05%.

The results showed that in 5 out of the 6 flocks there was a strong link between microbiota of the breeder and that of the offspring. As broilers aged their whole microbial profile became more similar to that of breeders, with the biggest differences around 21d. Looking at individual bacteria differences, there were on average only 3 bacteria significantly different between breeders and hatched chicks, 4 with broilers at 21d and 6 bacteria probes differences with broilers at 32d. This general pattern included more *Streptococcus* and *Clostridium* species in breeders compared to offspring, while more *Bacteroides* and *Lactobacillus* were found in broilers compared to breeders. Although these results demonstrate a link between breeder and offspring broiler microbiome, it is still expected that this connection is mostly done via the environment since they all belong to the same integrated poultry operation. At least under these conditions it was demonstrated that the microbiome connection between breeders and their offspring flocks is not lost. In one flock, higher *Salmonella* in breeders was followed by significantly higher *Salmonella* at hatch, which could indicate vertical transmission. The flock that did not follow the usual pattern of microbial maturation was the youngest breeders (35wks). That was also the only breeder flock with high levels of *Lactobacillus* and *Enterococcus*, though these differences did not affect the offspring performance of this flock. Therefore, we conclude that in an integrated poultry operation there was a strong link between breeder and offspring microbiome. This link can be positive or negative depending on the microbial profile of the breeders. Since the offspring flock that deviated from breeder microbiome had equivalent good performance, it appears to be possible to change broiler microbiome when breeder profile is not desired while maintaining broiler performance.

Use of aromatase inhibitor as an alternative for contraception in laying quails

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Several techniques are used to increase the ovulation rate and improve egg production as the bird ages. The objective of this study was to investigate the effect of aromatase inhibitor, letrozole, on layer quails. Laying quails aged 5 weeks were treated twice during egg production period with letrozole. Blood ovarian steroids: estrogen, progesterone and testosterone levels were measured. Body weight, egg production and egg quality characteristics were also studied. In letrozole-treated quails the blood estrogen level dramatically decreased while testosterone and progesterone increased. Letrozole induces complete cessation of egg production within day 4 after administration. Letrozole synchronized the onset of egg production and induces double-yolked eggs 9 days after withdrawal. The present study suggests that although administration of letrozole can hamper quail reproduction, but it can be used as anti-ovulatory drugs for induction and synchronization of egg production in laying quail.

Posters

Incubation traits and embryo development in two broiler breeder pure lines divergently selected on their energetic status

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Selection for meat traits has been accompanied by significant physiological changes such as those observed in reproductive performance and embryo development. Impact of a divergent selection on the breast meat ultimate pH, which reflects the level of glycogen reserves, is under study. Incubation traits and embryonic development were evaluated in the high pH line (pHu+, representing the lower energy status) and low pH line (pHu-, representing the higher energy status) after 11 generations of divergent selection from a base population corresponding to a commercial grandparental female broiler line.

Eighty females for the pHu+ and pHu- lines were housed in controlled environment from 20 to 40 weeks of age. Artificial insemination was performed twice a week from 28 weeks of age. For each line three batches of a minimum of 300 eggs each, laid between 30 and 34 weeks of age, were incubated in a semi-commercial incubator and hatcher. On the 7^{th} day of incubation, eggs were candled and all undeveloped eggs were removed and were opened to evaluate true fertility and determine the age of death of embryos in order to record the early embryonic mortality; the middle and late mortality were evaluated with the same procedure at day 14 of incubation and on the unhatched eggs, respectively. Hatchability was recorded at the end of each incubation. The embryonic development was evaluated on eggs from the 30^{th} week of age in a dedicated incubation, by recording the embryos wet body weight from day 4 to day 21 of incubation. From these data, the embryonic growth pattern of the two lines was estimated using the Gompertz model. The goodness of fit of the models were assessed using R^2_{adj} and differences between the two lines established in terms of inflection points of estimated maximum daily weight gain of the embryos.

High true fertility (TF) values were measured in both lines. The mean TF value was 86.90% for pHu+ and 89.40% for pHu-. Mean Hatchability showed a difference of 3.61% between the two lines. Embryo mortality occurred mainly between 24 hours and day 4 of incubation and during the hatch period, from day 18 to 21. Differences in embryo mortality profiles were observed: a higher early mortality was registered in pHu+ line whereas a higher late mortality was observed in pHu- line. In the embryonic growth models, differences were observed in terms of inflection points and in the estimated maximum daily body weight gain of the embryos from the two different lines.

In conclusion, pHu- line, which represents the higher energy status, had the best reproductive and incubation traits compared to pHu+ line, confirming that divergent selection on the meat ultimate pH has determined physiological changes in the reproductive performance, embryo development and viability between the two lines. These observations pave the way for future physiological and genetic studies to evaluate the contribution of energy status in terms of improving reproductive traits.

Effect of some organic additives to semen extender on rooster sperm quality after 6 hours of storage

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There are many extenders of different compositions, sometimes with special additives, both commercial and developed by individual investigators, that can be used for short (liquid) or long (in a frozen stage) term storage of chicken semen. Nevertheless, problems with natural mating of broiler breeder flocks, as well as the growing interest of consumers in a new, original poultry products means that interest in bird reproduction by artificial insemination and developing more effective extenders, is not diminishing.

We aimed to investigate the effect of enrichment of EK extender (Lukaszewicz, 2002) with some natural additives on chicken sperm characteristics after 6 h storage at 4°C. Pooled ejaculates were collected from 10 meat type roosters (HUBBARD FLEX), twice a week by dorso-abdominal massage. Males were kept in individual cages and controlled environmental conditions; water was provided *ad libitum*, food - 130 g/day/male. Freshly collected semen sample was divided into nine parts: 1) net semen; 2) diluted in 1:2 ratio with EK extender; 3) EK + 200 mg/ml of lyophilized quail egg white (LQEW); 4) EK + 100 mg/ml LQEW; 5) EK + 50 mg/ml LQEW; 6) EK + 100 mg/ml of lyophilized quail egg yolk (LQEY); 7) EK + 50 mg/ml LQEY; 8) EK + 25 mg/ml LQEY; 9) EK + 100 mg/ml of lyophilized whey of cow colostrum. In the diluted semen samples (after 15 min and 6 h storage) sperm morphology (on the basis of nigrosine-eosin histological smears; at 1250x magnification, Nikon Eclipse E100 light microscope), and motility (with Sperm Class Analyzer, version 5.1, Microptic, Barcelona, Spain) was determined. Twelve replications were made.

Although, the addition of tested organic substances had positive effect on sperm quality comparing to net semen or sample diluted exclusively with EK extender, none of them prevented an adverse changes after 6 hours of storage. In all extenders the decrease in percentage of total live and live normal sperm and in their motility was observed, but the significance of these decreases was dependent on the particular extender. The most beneficial effect on sperm morphology after 6 hours storage was found in EK supplemented with 100 mg/ml of LQEY (90.1% of total live and 68.0% of live normal sperm) and next, in EK with 200 mg/ml of LQEW (87.5% and 68.2%, respectively), while sperm motility was the highest (77.1%) in EK with 50 mg/ml of LQEW.

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Multigenerational effects of heat manipulation during embryogenesis on body temperature and growth in broiler chickens

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Fast-growing broilers are heat-sensitive animals, especially at slaughter age. Thermal manipulation during embryogenesis consisting in increasing incubation temperature of eggs up to 39.5°C and relative humidity (RH) to 65% for 12h/d 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 5 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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Rapid experience-dependent 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.

Effect of flock age, frequency of turning and SPIDES during storage on embryonic development, and hatchability of long stored eggs

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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 d 5 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 6 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 4 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 4 times daily compared to no turning during storage due to lower late embryonic mortality ($P<0.05$). This effect was more evident for older flock. SPIDES increased hatchability and reduced embryonic mortality and second grade chick 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 4 times and given one SPIDES treatment at 5 days of 14d storage in both flock ages.

Keywords: hatching eggs, embryonic development, SPIDES, turning, flock age, hatchability