ABSTRACTS

IFRG-Meeting and PDP-Workshop

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Session 1
Data analysis, in-ovo sexing

Keynote: Surviving the data mountain

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Hatcheries collect large quantities of data to monitor performance and to make sure equipment are operating within set operating limits. These data are a valuable resource for the hatchery, and can be used identify problems and areas where improvements can be made. To achieve this it is essential that data are handled and analysed correctly.

Good data analysis requires data to be stored in a database, effectively all the data to be analysed is in a single table or Excel sheet consisting of continuous columns and rows. The quality of the data analysis will also depend on the quality of the data: errors in the data will result in errors in the analysis. One of the largest tasks when undertaking a data analysis is the validating of the source data and this can be done using a variety of methods: (1) plotting all the data points to look for extreme values; (2) sorting or filtering data to look for values outside expected ranges; (3) check for consistent naming.

Reviewing data is an important management function and should be done routinely. The use of Excel Pivot Tables and Charts can be extremely powerful tools to help organise data and present the information in a meaningful way. Creating dashboards, where key management data are presented in tables and charts in a single view, can also a powerful way of monitoring the performance of the hatchery. Key to presenting data is that it shows performance against targets and that it highlights problems.

Statistical analysis can be a very powerful method for truly understanding the factors that are affecting performance. The advantage of statistical methodology is that it can include many factors at the same time within the analysis so that each factor can be evaluated when all the other factors have been accounted for. It is often stated that a statistical analysis can measure the pure effect of a given factor on performance. There are many techniques and computer programs that allow the user to carry out a statistical analysis, but all require the user to have some knowledge of statistical methodology. The main methodologies used to analyse hatch data are multiple regression, standardized least squares and general linear models. Potential pitfalls when running a statistical analysis are that factors being investigated are not independent of each other: class variables are confounded or continuous variables are highly correlated with each other.

The other type of data analysis that may need to be undertaken in a hatchery are results from a field trial, for example comparing the performance of two incubator settings. Running a successful hatchability field trial has three key requirements:

1. As much as possible make sure everything, other than the factor being investigated, is as equal as possible. For example if comparing two incubation settings make sure the eggs in the two test setters are from the same flocks and have the same egg storage.
2. Use lots of eggs. To detect a 2% hatch gain will require approximately 4,800 eggs per treatment and to detect a 1% hatch gain will require approximately 20,000 eggs.
3. Repeat the test several times, typically 3 or more, to make sure the results are consistent. When repeating a trial, if possible change which incubator is used for the control and test treatments.

In-ovo sexing of laying hen hybrids using endocrine analysis of the allantoic fluid

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In Germany, about 40 million day-old male chicks are culled each year, predominantly because of economic reasons. This is unacceptable with regard to animal welfare and ethical aspects. Taking current political decisions on this topic into account, alternatives to the culling of male day-old chicks are urgently required. *In ovo* sexing of laying hen hybrids represents one potential approach to solve the problem. The embryonic gender is determined before hatch and the eggs containing male embryos can be subsequently eliminated, preferably before the onset of embryonic pain perception.

The aim of this study was the development of a reliable method for *in ovo* gender identification with the help of sex-specific differences in the hormone concentration of the allantoic fluid of seven to ten day old chick embryos. Furthermore, the influence of gender identification on embryonic development, hatching rate, rearing as well as production performance of the adult hens up to 33 weeks of age was analysed.

Within a first study 750 eggs of the brown layer-hybrid Lohmann Brown (LB) were used. Withdrawn allantoic fluid was analysed via enzyme immunoassays (ELISA) for 17β-oestradiol (E_2), oestrone sulphate (E_1S) and testosterone. With regard to E_2 and E_1S, significant ($P < 0.01$) sex-specific differences were observed in nine and ten day old embryos. Testosterone on the other hand displayed no gender-related variances. Statistical analysis showed that the analysis of E_1S allows an earlier and more accurate sexing than the E_2-assay.

In a second study allantoic fluid of day 8 + 4 h (n = 2420) and day 9 + 4 h (n = 2850) old LB embryos as well as n = 150 day 9 + 4 h old embryos of the white layer-hybrid Lohmann Selected Leghorn (LSL) was analysed. For day 8 + 4 h old embryos the sex was correctly identified in 84 %. The accuracy of gender prediction increased for day 9 + 4 h old embryos up to 98 % (LB) and 100 % (LSL). Subsequently, 150 animals of the experimental group and 80 animals of the control group were reared for a period of 17 weeks. In the following production performance trial, 120 hens from the experimental and 60 hens from the control group displayed no significant differences in egg production, egg weight, bodyweight and feed consumption up to 33 weeks of age ($P > 0.05$).

These results demonstrate that an early *in ovo* sexing of chicken embryos is possible via the measurement of E_1S in the allantoic fluid. As the hatching rate is only marginally reduced and the production performance of adult hens is not affected, the described technique fulfils the basic requirements for an ethical alternative to the culling of day-old male layers.

Session 2
Embryo nutrition and mortality

The nutritional and physiological role of the yolk membrane during incubation

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During the 21 days of embryonic development, the chick embryo utilizes essential nutrients from the yolk for tissue growth, extra-embryonic tissue development and for its energetic needs. The yolk, deposited by the hen into oocytes, is the almost exclusive source of nutrients for the developing embryo, because it contains macromolecular complexes comprising lipids, proteins, vitamins, minerals and other essential micronutrients.

Throughout incubation the yolk membrane, which covers the yolk, goes through intensive process of proliferation, differentiation and also degradation. Ultrastructural studies have provided information on the dynamics of the morphological transformations of the yolk membrane upon transition of the area vitellina to the area vasculosa. During this transition, the apical part of endodermal epithelial cells (EEC) acquires the typical characteristics of polarized epithelial cells with villi invaginations into the yolk. Weight and absorptive area of this tissue increase by approximately 30-fold between E5 and E17 and then decreased by 3-fold between E17 to E21.

Transcriptome analysis of large-scale patterns of gene expression (at E13, E15, E17, E19, and hatch), clustering and functional annotation of 3547 genes as well as histological sectioning revealed that the yolk is enveloped with a tissue which plays different roles to support or replace the functions of several organs that have not yet reached their full functional capacity. This tissue has a similar functional role as the intestine in digestion and transport of nutrients, as liver in producing glycogen and plasma carrier proteins, and as bone marrow in synthesis of blood cells.

Expanding knowledge on developmental, nutritional and molecular processes in the yolk will contribute to our understanding of incubation period which is about 35% of the chicken lifespan.

The effect of nutrient profiles in egg yolk on embryonic survival ability in laying hens

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Low hatchability negatively affects productivity and animal welfare in the poultry industry. About 8% of chicken embryos die before hatching each year; the value is much higher in turkeys. Embryonic viability is influenced by a series of factors such as nutrition, hatching technology, egg quality and genetics. The nutritive components of the yolk are influenced by environmental and genetic factors and could affect the embryonic survival ability. The main goal of this study was to determine metabolite profiles in the egg yolk and to assess possible associations with hatchability.

A large number of hatching eggs were collected from 4 different lines (commercial white- and brown layer lines and experimental unselected lines). Based on estimated breeding values of hatchability traits in hens of aforementioned lines, 1073 egg yolk samples were collected to determine metabolite profiles using gas chromatography–mass spectrometry. A total number of 105 different metabolites known in egg yolk, including fatty acids, amino acids, carbohydrates, steroids, glycerides, vitamins and organic acids were detected. The estimated heritability for different metabolites was in the range between 0 and 70%.

Significant differences were found between different lines. Compared to white layers lower amounts of saturated fatty acids and monounsaturated fatty acids were detected in brown layers' egg yolks, whereas the content of polyunsaturated fatty acids, was higher in brown layers. A significant association between embryonic survival ability and the polyunsaturated fatty acids arachidonic acid and docosahexaenoic acid was found. These fatty acids are essential for the development of the embryonic brain and nervous system in precocial birds. Furthermore, a significant positive association was observed between embryonic mortality and palmitoleic acid and it's precursor palmitic acid, which are known to influence insulin content and glucose metabolite pathways during embryonic development.

Analysis of embryo mortality and 'clear' Bilgoraj goose eggs after incubation and candling

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The Bilgoraj goose is unique to Poland. Like other geese its egg production season is short, producing 45-55 eggs per season and its fertility somewhat low (70-75%). Consequently, only 20-30 Bilgoraj goslings are hatched out per goose each year. In accordance with the Genetic Resources Conservation Program of Poland, which has a stated goal of maintaining bird population at the level to keep adequate genetic variation and avoid inbreeding, we are interested in improving the reproductive efficiency of the Bilgoraj goose.

The goal of the following study was to determine the status of clear eggs, that is unfertilized or early dead, at 8-day candling and status of eggs containing dead embryos after incubation during the first reproductive season of Bilgoraj goose.

The eggs were candled twice – at 8th and 26th day of an incubation period of 29 days.

Following breakouts, two peaks of embryo mortality were observed. The first peak was during days 1-3 of incubation; the second peak was on days 24-29. There was also a small peak during days 14-16. In different days of incubation the embryo mortality ranged from 14.72% in day 2nd to 0.11% in day 11th. The difference between true fertility rate and candling fertility, which we refer to as correction of fertility rate, ranged from 2% to 20% in some sets of eggs and increased at the end of egg producing season.

The early embryo mortality may be caused by many factors, among others on incorrect storage and incubation conditions. The peak of embryo mortality in the middle of incubation is most likely associated with improper closing allantois and increased embryo metabolism.

The increased number of dead embryos at the last few days of incubation may be result of changing embryo respiration - from the allantois to lung respiration and its increased demand for oxygen.

The increase of candling fertility at the end of the geese reproductive season may be caused by decrease in biological value of eggs or lower sperm quality.

The effect of increased concentration of carbon dioxide during early incubation on albumen characteristics, embryonic mortality, and hatchability

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Three experiments were conducted to determine the effects of increased CO_2 concentration during early period of incubation on albumen height and pH, embryonic mortality, and hatchability. Eggs from 31, 39 and 35-week-old commercial flocks of Ross 344 x Ross 308 broiler breeders were incubated under either standard ventilated conditions for the entire incubation or increased CO_2 levels during the first 2 or 3d of incubation (non-ventilated) in Experiments 1, 2 and 3, respectively. In all cases eggs were stored for 1 d at 18ºC and 75% RH before use. A total of 1680 eggs were placed in twenty-eight 60-egg trays and set in two laboratory incubators and the CO_2 level was increased gradually from the beginning of incubation onwards to reach 0.50% at 48 h or 0.80 % at 72 h by manual injection of CO_2 into an air tight incubator in Experiments 1 and 2, respectively. In the control incubators, CO_2 concentration remained below 0.1%. After the termination of the CO_2 injection, all eggs from both groups were mixed and equally distributed among the two incubators and the level of CO_2 was the same as it was in ambient air. Before the start of incubation, 90 eggs of the same weight were selected and 30 eggs were opened for albumen height and pH measurements. The remaining 60 eggs were divided and set in the two incubators to be utilized for measurements at the end of the CO_2 injection in experiments 1 and 2. In Experiment 3, eggs were set in a commercial Petersime BioS model of setters and hatchers. During the first 3 d of incubation CO_2 was gradually increased to reach 0.80 % at 72 h naturally (not ventilated). In the control incubator, CO_2 concentration remained below 0.1% (ventilated). After 3d of incubation, the level of CO_2 was slightly reached to 0.30% through to 19 d of incubation in both incubators in experiment 3.

Albumen height was not affected by CO_2 treatment but significantly decreased albumen pH at 2 d or 3 d in experiment 1 and 2 respectively($P<0.05$). Greater CO_2 level during early incubation reduced fertile hatchability due to increased early embryonic mortality in 3 experiments ($P<0.05$).

The differences in pH might provide one explanation why increased CO_2 during early incubation result in increased early embryonic mortality. These data indicated that at the beginning of the incubation, ventilation was necessary to prevent the increases of CO_2 concentration for optimum hatchability results.

Session 3
Fertility and storage

Keynote: Impact of broiler egg storage on relative expression of blastoderm genes

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Cool temperature storage of eggs prior to incubation is a frequent practice by commercial broiler hatcheries. However, continued storage beyond 7 days leads to an increase in early embryonic mortality due to the progressive increase in embryonic cell death. The cellular and molecular basis for this phenomenon is not known.

A customized commercially available kit (Qiagen) was used to analyze the expression of a panel of genes associated with oxidative stress, genetically programmed cell death (apoptosis), and fatty acid metabolism from RNA extracted from embryos isolated from unstored eggs, eggs stored for 21 days, and eggs stored for 21 days but subjected to three, 4 hr sessions of warming (37oC) on days 6, 12 and 18 of storage (SPIDES). The SPIDES treatment has been shown to increase the number of viable embryos by 8-10% after storage when compared to untreated stored eggs. By assigning the gene expression values of the embryos from unstored eggs as zero, the relative expression of the 29 genes examined in the two egg storage groups and control group were compared.

The expression of fatty acid binding protein was significantly greater in the SPIDES treatment compared to either the control or the stored non-SPIDES embryo. In contrast, glutathione peroxidase, an anti-oxidant, was significantly greater in the non-SPIDES embryos compared to either the control or SPIDES embryos. No significant differences in the three treatments were observed for the remaining 18 of the genes examined. The data from a second trial that included blastoderms from unstored eggs incubated for 10 hr (blastoderms reached the same developmental stage as the SPIDES eggs) are currently being summarized.

Once the molecular pathways leading to embryo mortality during egg storage are understood, it would be a matter of influencing the direction of that pathway though temperature manipulation, diet, or genetic selection to minimize embryonic mortality.

Effects of different conditions of storage on egg components and blastodermal quality and high temperature environments

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Egg quality is a general term that relates to various standards that are imposed on eggs. This quality usually embraces a range of quality characteristics such as shell colour, albumen quality amongst others. It is therefore necessary to store eggs properly to avoid or reduce the rate at which the quality declines. Egg quality has also shown to be an influencing factor in hatchability and chick quality in general.

Therefore, studies were conducted to determine the influence of storage temperature, condition and duration on egg quality characteristics, shelf life and blastodermal size.

A total of one hundred and ten eggs were used for two experiments. In the first experiment, a total of 60 table eggs were divided into two treatments of oil and non-oil coating. Eggs (n = 10) for each treatment were stored for either 0, 2, 4, 6, 8 and 10 days at ambient temperature. A Completely Randomized Design (CRD) in a 2x5 factorial arrangement was used. Experiment two comprised of two treatments of cold storage (18oC) and ambient temperature storage (23-26oC). Fertile eggs under each storage condition were stored for 1, 3, 6, 10 and 14 days. Parameters measured included proportions of yolk, shell, albumen and blastodermal size. Data was analysed using the SAS Proc. GLM procedure ($P<0.05$).

The results showed that in experiment 1, shell thickness was affected by oil preservation. Yolk weight and Haugh unit were significantly affected by storage duration. The Haugh unit decreased as the storage days increased. In experiment 2, the egg weights were not affected significantly by storage conditions but were significantly affected by storage duration and interactions between storage condition and storage duration. The blastoderm size decreased significantly in cold temperature compared to ambient temperature and increased significantly as the day of storage increased. In a similar way, the yolk weight increased as the day of storage increased.

Based on the research findings it was concluded that in table eggs egg quality as measured by Haugh unit is not affected by oil preservation but quality decreases with increasing storage duration. In fertile eggs while the blastoderm quality on both dependent on both storage temperature and duration, the egg components of yolk, shell and albumen were much dependent on storage duration.

Effect of SPIDES and slow warming profile on hatchability of long stored eggs

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Egg storage beyond 7 days decreases hatchability. It is known that Short Incubation Periods During Egg Storage (SPIDES) or a slow warming profile at the start of incubation can reduce the negative effect on hatchability when eggs are stored. In the current study, the effect of SPIDES or a slow warming profile on hatchability was investigated when Ross 308 eggs from a 36 week old breeder flock were stored for 21 days (n=1200). Hatchability was compared to a short (4 days) and long (21 days) storage control treatment.

SPIDES was performed at day 11 and 17 (2xSPIDES) or at day 4, 8, 13, and 17 (4xSPIDES). Eggs were warmed to an EST of 37.8°C using a linear warming profile of 10 hours. When an EST of 37.8°C was reached, eggs were cooled down inside the incubator to an EST of 25°C. Afterwards eggs were returned to the storage room where the air temperature was maintained at 18°C.

During the slow warming profile at the start of incubation, EST increased linearly from 21°C to 29.4°C in 5 hours and from 29.4°C to 37.8°C in another 17 hours (WP 5-17), while the warming profile of the short and long storage control treatments was WP 5-5.

Hatchability of the short storage control was 53.5% higher than of the long storage control (89.5% vs. 36.0%). In comparison to the long storage control, WP 5-17 improved hatchability by 18% and 4xSPIDES treatment improved hatchability by 27.2%. 2xSPIDES improved hatchability by 48.2% and was equal to hatchability of the short storage control (84.2% vs. 89.5%; $P<0.001$).

It can be concluded that 2xSPIDES used in the current study prevented any decline in hatchability during 21 days of storage. WP5-17 and 4xSPIDES resulted in higher hatchability than the long storage control. However, hatchability of WP5-17 and 4xSPIDES was lower than of the short storage control due to a higher embryonic mortality during the first two days of incubation for WP5-17 and due to a higher embryonic mortality between days 3 and 9 of incubation for 4xSPIDES.

Session 4
Incubation temperature and hatching time

Increasing and decreasing incubation temperatures during embryonic myogenesis influences muscle growth and energy metabolism in broiler embryos

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In ovo embryogenesis in broiler can be modulated by alteration of external factors, like the incubation temperature, as shown in several publications. Embryonic myogenesis in broiler is characterized by the formation of primary and secondary myotubes during embryonic day (ED) 3 to 8 and ED 8 to 14, respectively and it was shown that increase of the incubation temperature during this period has an impact on the growth of the embryos and animals post-hatch (Maltby et al., 2004; Hammond et al., 2007; Janisch et al., 2015).

Assuming that the described effects of the in ovo temperature alteration on the muscularity were related to the metabolism of the embryo, in the present study the mitochondrial respiratory activities and the activities of enzymes of the energy metabolism within the breast muscles of differently incubated embryos were analysed directly after treatment (ED 10, ED 13). Therefore eggs of a commercial fast growing broiler line were incubated at higher (38.8 °C), lower (36.8 °C) or normal temperatures (37.8 °C (Control)) between ED 7 and 10 or ED 10 and 13. Weight characteristics as well as mitochondrial respiratory (MRA) and enzyme activities of the breast muscle samples of the ED 10 and 13 embryos were analyzed.

Temperature increase results in higher body, liver and heart weight on ED 10 and higher body weights on day 13 compared to at 36.8 °C incubated embryos. The same differences could be determined for the MRA on days 10 and 13, the activities of the lactate dehydrogenase and cytochrome oxidase on day 10 and the glycogen phosphorylase, phosphofructokinase, cytochrome oxidase activities on day 13. Control results were variable differing from the high or low temperated samples, from both or none of them.

The data show that the temperature effect on the embryo growth was related to the muscle metabolism probably due to direct alterations of the MRA and enzymes and/or general change of embryo activity/movement.

The impact of raised incubation temperature on hatch, chick quality and broiler performance

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It is well recognized that eggs which have been overheated during incubation do not hatch as well as they would have if incubated under more appropriate conditions. However, there is less information available about the direct impact of high incubation temperatures on the performance of the chicks after they hatch.

Two experiments were conducted at the Aviagen product development center in the USA to investigate the effect of increased eggshell temperature during mid to late incubation on hatchability, chick quality, yolk sac, heart, digestive organs, and broiler performance.
Three treatments were imposed in each experiment. Egg shell temperatures were recorded using Gemini data loggers feeding to a wireless broadcast system that could be interrogated in real time. Incubator conditions were changed as necessary to maintain the desired egg shell temperature. From set to day 10, all the eggs were held at the same eggshell temperature, 100.0°F (37.8°C). Treatment 1 was the control, and eggshell temperature was set at 100.0°F all the way through to transfer. Treatment 2 was set at 101.5°F (38.1°C) and Treatment 3 at 103.0°F (39.4°C) from day 11 to transfer.
In the first experiment, 2,310 Ross 308 broiler hatching eggs from a 38 week old flock were used in each treatment. The eggs were taken through to hatch, and full hatch data were recorded. Chick quality was evaluated after takeoff and the chicks placed as broilers, reared to 38 days. The treatment of 100.0°F (37.8°C) eggshell temperature throughout incubation period resulted in better hatchability, a higher percentage of first quality chicks, a higher body weight at 38 day, and improved FCR at 38 day when compared to incubation with eggshell temperatures of 101.5°F (38.6°C) or 103.0°F (39.4°C).
In the second trial, 1,815 Ross 308 and 1,815 Ross 708 broiler hatching eggs from a 39 week old flock were placed for each treatment. Full hatch data were collected; chicks were evaluated for chick quality and then placed as broilers, grown through to 53 days. The treatments were the same as in the first trial.

Eggs that were hatched using a 100.0°F (37.8°C) eggshell temperature throughout the incubation period had better hatchability and body weight at 53 day when compared to incubation with eggshell temperatures of 101.5°F (38.6°C) or 103.0°F (39.4°C). The chicks on the control incubation treatment had bigger hearts as a percentage of yolk-free body weight than those incubated on the two hotter treatments.

Chicks that were hatched after a constant 100.0°F (37.8°C) eggshell temperature throughout the incubation period had fewer red hocks, bad navels, lower residual yolk weight, and better liveability at 53 days compared to the high temperature treatments on both Ross 308 and 708.

In both trials, hatchability was as expected when eggs were incubated too hot. Broiler performance was impaired when higher egg shell temperatures were imposed from 11-18 days incubation.

Effect of temperature during the last week of incubation on embryonic development and chick quality

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Incubation conditions need to be adjusted to meet embryonic requirements to obtain optimal incubation results. Temperature drives embryo development, growth, and hatchability; therefore it has an impact on chick quality and performance.

In the current study, the effect of temperature applied from embryonic day (E) 14, E16, or E18 until hatch on embryonic development and chick quality was investigated. A total of 2,850 first grade eggs of a 43 week old Ross 308 broiler breeder flock were selected on egg weight between 62 and 65g. Until E14, eggs were incubated at a constant eggshell temperature (EST) of 100F. At E14, E16, and E18, eggs (240 at E14, 210 at E16, and 180 at E18) were moved from a control treatment of 100F to one of 3 other climate respiration chambers in which EST was maintained at 96F, 98F, or 102F. At E14, E16, E18, moment of internal pipping (IP), external pipping (EP), and hatch, and weights of yolk free body mass (YFBM), yolk, heart, liver, stomach, spleen, bursa, and intestines were determined. Organ weights were expressed as percentage of YFBM.

At E18, an effect of EST was found for YFBM ($P=0.008$), relative heart ($P<0.0001$), and relative liver weight ($P<0.0001$). At 96F, YFBM was lower and relative heart and liver weights were higher compared to all other treatments. At IP, an effect of EST was found for yolk weight ($P<0.001$), relative heart ($P<0.0001$), and relative liver weight ($P<0.001$). At 96F, yolk weight was lower and relative heart, and relative liver weights were higher compared to all other treatments. At EP, an effect of EST was found for yolk weight ($P<0.0001$), relative heart ($P<0.0001$), and relative liver weight ($P<0.0001$). At 96F, yolk weight was lower compared to 100F ($D=0.69$g) and 102F ($D=0.99$g). At 96F, relative heart weight was higher compared to all other treatments. And at 102F, relative liver weight was lower compared to all other treatments. At hatch, an effect of EST was found for YFBM ($P<0.001$), yolk weight ($P<0.0001$), relative heart ($P<0.0001$), and relative liver weight ($P<0.0001$). At 96F and 98F, YFBM and relative liver weight were higher compared to 100F and 102F. At 96F, relative heart weight was higher compared to all other treatments. At 102F, yolk weight was higher compared to all other treatments.

It can be concluded that an EST of 96F and 98F during the last week of incubation increased chick development, mainly expressed by the higher relative heart weight at IP and EP, and the lower yolk weight at IP and EP. In addition, an EST of 96Fand 98F results in a higher YFBM at hatch than 100F and 102F, which indicates that chick development is optimized when eggshell temperatures are below 100F from E14 onwards.

Relationship of hatch time and posthatch holding time in the hatcher to subsequent broiler live performance

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This study investigated the effects of broiler chick hatching time and delayed placement after hatching by extending hatcher holding time on subsequent live performance.

Broiler hatching eggs were obtained from commercial flocks of Ross 344 males and Ross 308 females at 29 and 30 wk of age in Experiments 1 and 2, respectively. Eggs were stored for 1-3 d at 18°C and 75% RH prior to setting and incubated in Petersime machines on 2 consecutive days under standard conditions as recommended by the manufacturer. A part of the eggs (6000) were incubated on the first day of set was identified as held pull treatment (HP) and other part of the eggs (6000) were incubated on the second day of set were identified as normal pull treatment (NP) in both experiments. The hatching period was divided into three hatch time groups and the Early hatch time was before 480 h (EH), the Middle hatch time was from 480 to 492 (MH), and the Late hatch time was after 492 to 510 h of incubation (LH) in two holding time groups. At 510 h of incubation (based on NP set date), all chicks were removed from the trays, counted, feather sexed, and transferred to poultry house in 4 hours in both Experiments. For each hatch group per holding time, chicks were assigned to 6 or 7 pens of 100 male and 100 female chicks for a total of 7200 and 8400 chicks within 2 holding time x 3 hatching time groups in Experiments 1 and 2, respectively. Chicks were group-weighted by pen at placement (0 d) and at 7, and 41 d of age. Feed consumption was determined at 7, and 41 d of age and FCR was calculated for 0-7 d, and 0-41d. Mortality was determined daily and EPEI was calculated as EPEI= [Live weight (kg) x Livability (%)]/[(Age of depletion (days)xFCRx10].

In this study, the primary difference between the HP and NP group was an additional 24 h holdover of the HP group during the hatching period. As expected that broiler chick BW was greater at placement in Normal pull chicks (NP) compared to Held pull chicks (HP) in both experiments ($P < 0.01$). However, this advantage disappeared by 7 d and average BW did not differ between the HP and the NP groups at 41 d in both experiments. As found for BW, holding time in the hatcher did not affect mortality, FCR and EPEI at 41d in both experiments ($P > 0.05$). Hatch time did not affect live performance at 41d in HP group in both experiments however in NP group, Live performance of Late hatch chicks was reduced compared to Early and Middle hatch chicks at 41d in two experiments.

These data indicated that, the HP groups that were held an additional 24 h in the hatcher and exhibited a lighter initial BW accompanied by a period of compensatory gain in BW through 41d, and no differences ($P>0.05$) in live performance occurred due to holding time in the hatcher.

Session 5
In-ovo photo and temperature stimulation

Characterizing developmental pattern of retinal and extra retinal photoreceptors in broilers following in ovo and post hatch photostimulation with different wavelength

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Many physiological processes related to reproduction, growth and development are influenced by environmental conditions. The influence of light spectrum on poultry growth and reproduction has been demonstrated in many studies. Green and blue lights photostimulations induced post hatch growth of meat type birds. In addition, broilers' growth has demonstrated as related to in ovo green light photostimulation as well. Birds perceive light through the retina and extra retinal sites, such as the pineal gland, olfactory bulbs and hypothalamus. The involvement of retinal and extra retinal photoreceptors on growth and development of meat type birds is unknown.

We examined the effect of different *in-ovo* and post-hatch wavelength photostimulation on mRNA green opsin gene expression in the retina and hypothalamus. Fertile broiler eggs were *in-ovo* photostimulated with 560 nm (green light), 480 nm (blue light), 660 nm (red light), and white light or incubated in dark conditions (control). After hatch each *in-ovo* treated group was sub-divided to 4 light treatments: green, blue, red, and white. All lights (in-ovo and post-hatch) were provided by LED lamps. Samples were taken from the hypothalamus and the retina, from day 10 of incubation (E10) to 12 days post hatch. Retinal and hypothalamic green opsin gene expression were measured by RT-PCR.

In-ovo photostimulation with green light elevated retinal and hypothalamic green opsin gene expression, while blue light was found to reduce it. Red and white light were also found to induce greater expression of the green opsin gene but to a lesser degree than the green light compare to dark control. When testing the effects of integrated photostimulation *in-ovo* and post-hatch, we found that post-hatch green photostimulation further increased the expression of green opsin gene, while using blue light further decreased this expression both in retinal and hypothalamic tissues. In other words, an additive and synergistic effect exists in response to integrated photostimulation with green and blue light. Furthermore, a positive correlation between green opsin gene levels and body weights was detected.

Thus, we suggest that monochromatic green light, causes an increase in green opsin expression which improves light perception that induces growth via behavioral and endocrine mechanisms.

The effect of *in-ovo* photostimulation with monochromatic green light during incubation on the expression of the somatotrophic axis in broilers

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Green light *in-ovo* photostimulation of broilers, caused elevation of body weight and muscle growth.

We studied the effect of *in-ovo* photostimulation with green light, on the somatotrophic axis gene expression in the hypothalamus, pituitary, liver and muscle, in order to understand the mechanism of growth acceleration.

Two hundred and forty broiler eggs (Cobb average weight of 63 g +\-3 g), were divided to 2 equal weight groups: one group was incubated at dark condition (Control), and the second was photostimulated with green light (560nm, 0.1 W/m2 intesity at egg shell level). At 10th days of incubation (E10) and every other day until hatch (E20), 14 eggs from each group were used. Eggs were opened and embryos were collected and weighed. Liver and breast muscle samples were harvested and weighed. Hypothalamus and pituitary were also sampled, without weighting. mRNA was extracted from the samples, and gene expression of somatotrophic axis was measured by RT-PCR. Statistical analysis was preformed, using One Way Anova, in JMP 7.

In-ovo photostimulation with green light caused increase in liver weight that was significant at hatching (day 20). In addition, *in-ovo* green light photostimulation caused an elevation in the mRNA gene expression of hypothalamic GHRH form E16 till hatching day (day E20), GH receptors from E16 to E18, as well as increased liver IGF-1 from E14 till E18. In the muscle, we found an increased mRNA gene expression of the IGF-1 from E14 till E18.

The results of this study, show increased mRNA gene expression in several of the somatotrophic axis components, including GHRH, GH receptors, and IGF-1, due to *in-ovo* photostimulation with green light. We suggest that the effect of *in-ovo* green light photostimulation, accelerating both body weight and muscle growth in broilers, is due to increase in the activity of the somatotrophic axis. These results also suggest that the effect of green light on muscle growth, is both direct (in the case of muscle IGF-1 expression), and indirect (in the case of hypothalamus GHRH, liver GH receptors and IGF-1).

Effect of cold incubation temperature and cold ambient temperature at start on performances, body temperature and health criteria in fast-growing broiler chickens

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High performances of broilers are associated with high sensitivity to their climatic environment. Rearing birds under low temperatures has severe economic and welfare consequences, such as decreased feed efficiency (particularly for chicks) and an increased occurrence of ascites syndrome in finishing broilers. Several strategies were proposed to increase the adaptive capacities of birds and consequently their robustness, especially regarding thermal exposure. Thermal manipulation during specific phases of embryogenesis was previously reported to decrease ascites occurrence posthatch and may be a way to improve the cold tolerance and robustness of broilers.

The objective of our study was therefore to explore the effects of the interaction of cold incubation temperatures and cold ambient temperatures during the first 21 days of rearing on performance and selected health parameters. Ross 308 eggs were incubated either in control conditions (37.7°C, C) or with cyclic cold stimulations (6h/d at 36.7°C from day E10 to E18 of embryogenesis, A1), or with two short acute cold stimulations (30 min at 15°C and 80% relative humidity at days E18 and E19 of embryogenesis, A2). These treatments were followed by postnatal exposure to standard rearing conditions (S, from 33°C at hatch to 21°C at 21-day-old) or continuously lower ambient temperature (L1, from 28°C at hatch to 21°C at 21-day-old) or exposure to cyclically lower ambient temperature (L2, oscillating between both previous thermal regimes). Chickens were reared 4 per cage until 3 days of age and individually until 21 days. Thereafter chickens were transferred to a single floor pen in standard conditions until 35 days of age. Treatments A1 and A2 did not alter hatchability as compared to control eggs with 94.8%, 95.1% and 92.3% of fertile eggs, respectively, or body weight or chick quality at hatch. Male body temperature at hatch was higher in A1 than in C ($P < 0.05$) whereas for females body temperatures were not different between groups. A higher occurrence of leg disorders was observed with the continuously lower ambient temperature (L1), whatever the incubation condition or the gender. No effect of incubation or postnatal treatment was observed on body weight at 0, 11 and 21 days in males. Ambient temperature L1 affected females by reducing body weight at 21 days, which was compensated at 35 days once transferred to standard rearing conditions. At 21 days, there was an interaction between incubation temperature and postnatal thermal conditions on feed conversion ratio (FCR) measured in males. In females, both A1 incubation and L1 postnatal temperature increased FCR.

In conclusion both cold incubation and cold ambient temperature at start changed performances and health of broilers, the effects depending on the gender.

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Improvement of body weight gain and feed conversion in laying-type cockerels of Lohmann Dual by short-timing temperature stimulation before hatching – a comparative study

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Laying-type cockerels or spring chicken cannot be reared economically. But routine culling of these day-old male chicks is more and more an ethical problem and to find alternative solutions is a great challenge. Lohmann Tierzucht bred the dual-purpose chicken (producing eggs and meat) in response to growing criticism of conventional practices in modern egg production. Recent research with birds shows that incubation climate may have a long-lasting influence on poultry performance up to the age of slaughter. In poultry embryos at the end of incubation, peripheral and central nervous thermo-regulatory mechanisms, as well as other body functions, are well developed, so that after mild temperature variations no negative side effects will be expected. Therefore the following study was carried out, to investigate whether short-term variation in incubation temperature during the last days of incubation have a long-lasting effect on performance, also in laying-type cockerels.

Methods:
2880 eggs (Lohmann Brown-LB/Lohmann Dual-LD) were incubated from days 1 to 17 under common incubation temperature (37^0C). From day 18 until hatching the eggs were sorted in hatch incubators with different temperature programs: 37^0C (control) and 1^0C over standard for 2 hours daily (38^0C: short-term warm stimulation). Chicks were sorted by sex and male cockerels were randomly distributed in 8 treatment groups (two origins of chicks-LB, LD; two hatch incubators; two different protein/energy-200 g crude protein/11 MJ AME_N/kg - low; 215 g/12 MJ - high) from day 1 to 70 of age. Data were analyzed via a three-way ANOVA (SAS).

Results and conclusion:
Growing performance of LD cockerels was significantly better compared to LB males (Table 1). Final body weight of LD birds was 1000 g higher and feed to gain ratio 10% lower. Short-term temperature stimulation during the end of incubation resulted in a 3.5% higher final body weight by LD cockerels.The daily feed intake and the feed to gain ratio was significantly improved through the increased protein/energy concentration of the “high” feed.

Table 1 Feed intake, final body weight (age of 70 days) and feed to gain ratio of cockerels

Genetic line	Temperature stimulation	Feed level	Feed intake g/bird/day	Final body weight, g/bird	kg feed/kg weight gain
LB	Control	Low	47.6	1336	2.568
	Control	High	43.3	1360	2.293
	Stimulation	Low	47.1	1336	2.544
	Stimulation	High	44.1	1374	2.309
LD	Control	Low	78.6	2432	2.299
	Control	High	71.4	2482	2.049
	Stimulation	Low	81.9	2558	2.275
	Stimulation	High	73.9	2528	2.070
P-values	Genetic line		<0.001	<0.001	<0.001
	Temperature stimulation		0.14	0.09	0.96
	Feed level		<0.001	0.45	<0.001

Broiler breeder age and temperature stimulation during last days of incubation: Effects on hatching performance and cardiac and muscular development

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The objective was to evaluate the influence of broiler breeder age and thermal stimulation during last days of incubation on hatching performance and cardiac and muscular development in chickens.

The experimental design was completely randomized in a factorial 2 x 2 with two breeder ages (30-wk and 60-wk) and 2 temperature incubation programs. 2,520 eggs/treatment were incubated in 15 trays (n =168 eggs), distributed in two commercial hatcheries with single-stage incubation. A hatchery served as control (37.2-37.4ºC) and in second one the temperature was further increased 1ºC (38.2-38.4˚C) for 4 h/day (16 to 19-days of incubation). For hatching performance, hatchability of fertile eggs, fertility, embryonic mortality and discard chicks (abnormalities, problems in navel, legs, beak, and belly) were determined in each replicate. One-day chicks (n=1,088) were sexed and housed in the same experimental design used in the hatchery, adding the factor sex (male and female), with 4 pens of 34 chicks pert treatment. During incubation (16 and 19-d), at hatch and at 7 and 42-d heart and breast muscles were analyzed.

Eggs from breeder with 30-wk had better results to hatchability (94.63%), fertility (94.02%), and waste (0.46%). Thermal stimulation had no significant effect on hatching variables. Breeder age affected weight of eggs and embryos (16 and 19 days) and chicks (hatch and 7-days), with heavier results from breeder with 60-wk. For heart morphometric, there was no significant effect on the relative weight of the heart in any of the ages analyzed. Before thermal stimulation, at 16-days of incubation embryos from females with 60-wk had thicker left ventricle wall. At 42 days there was an interaction between breeder age and sex of chicks for the left ventricular wall thickness, in which males from young breeders had higher left ventricular thickness, while to chicks from 60-wk broiler breeder that effect was not significant. For muscle development, breeder age and thermal stimulation had interaction for the relative weight of breast muscles in chicks with 7-days. Chicks from oldest breeder and incubated without thermal manipulation showed higher breast relative weight when compared with chicks from youngest breeder. In chicks with thermic stimulation during incubation this effect was not observed. Sex of chicks influenced number of muscles cells/area of breast muscle at 7 days, with more cells in males' muscles samples. Breeder age affect the fiber diameter, with biggest cells in chicks from older breeders. There was no effect of breeder age, thermic stimulation or sex in relative breast weight at 42-d.

We conclude that thermal stimulation in last days of incubation affects muscle development of broilers and these effects should be considered in association with breeder age.

Session 6
Influence of incubation and post-hatching factors on bone and leg development

Broiler breeder age and temperature stimulation during last days of incubation: Effects on bone development and productive performance

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The thermoregulatory system of birds starts its development during embryogenesis and can be influenced and regulated by thermosensitive neurons located in the hypothalamus. Variations in temperature during incubation could influence the maturation of this system with consequence to embryo and chick development. This study investigated the effects of thermal manipulation, breeder age and sex in bone development of broiler chickens at 16 days of incubation to 42 days of age.

The experimental design was completely randomized in a factorial 2 x 2 with two breeder ages (30-wk and 60-wk) and 2 programs in temperature incubation. 2,520 eggs/treatment were incubated in 15 replicates (n = 168 eggs), distributed in two commercial hatcheries with a capacity of 120,960 eggs and single-stage. A hatchery served as control (37.2-37.4ºC) and in second one the temperature was further increased to 38.2-38.4˚C for 4 h/day (16 to 19-days of incubation). Chicks (n=1,088) were sexed and housed in a factorial 2 x 2 x 2 (breeder age, temperature program and sex), with 8 treatments and 4 replicates of 34 birds. Before (16-d) and after (19-d and hatch) thermal stimulation 8 birds per treatment were fixed in formaldehyde 10% in order to identify calcified bone tissue and obtain the total and calcified area length of femur and tibiotarsus.

Breeder age influenced the length of bones of embryos at 16-days, with longer bones in embryos from breeder of 60-wk. Thermal stimulation influenced the percentage of femoral calcification and the length of tibiotarsus at 19-days of incubation, with higher values to embryos from control group. The increment in temperature between 16 and 19 days of incubation influenced the physiology of embryos with negative impacts on bone development of femur and tibiotarsus. Seedor index, breaking strength and percentage of ashes were evaluated at 1, 7 and 42 days. Chicks from breeder with 60-wk had high Seedor index values to femur. Males had higher Seedor index values, breaking strength, diameter of the medullar region and cortical thickness than females, while females had higher values of ash percentage. Thermal manipulation and breeder age did not influenced feed intake, feed conversion, or weight gain at 1-21 days and 22-42 days and carcass yield at 42 days. For carcass yield, males had biggest breast, leg cuts and weight than females.

In conclusion, breeder age and thermal stimulation can affect bone development in broiler chick, but these effects were not significant after hatch and thermal stimulation in last days of incubation did not affect the broiler chickens productive performance.

Effect of lighted incubation from set till hatch on broiler leg bone development

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Hatcheries incubate eggs in complete darkness, while hatching eggs are regularly exposed to light in a natural situation. It can be speculated that light during incubation will influence bone development through the pathways of melatonin (involved in bone development with a rhythmic darkness-dependent release pattern) and increased embryonic activity. The present experiment aimed to investigate effects of light schedule throughout incubation on leg bone development during embryonic development, at hatch, and in later life in broiler chickens.

A total of 744 Ross 308 eggs of a 40 week old breeder flock were incubated from embryonic day (E) 0 till hatch at 1 of 3 light schedules: continuous darkness (24D); 12 hours of darkness, followed by 12 hours of light (12L:12D); and continuous light (24L). Eggshell temperatures were maintained at 37.8°C throughout. From E6 until E14, 10 embryos per measurement, per treatment (N = 270) were removed from the incubator daily for measurement of ossification of the femur and tibia through histological staining. 50 chicks per treatment (N = 150) were sampled within 3 hours after hatch to determine leg bone measurements (tibia and femur weight, length, width, and depth). 108 chicks per treatment were moved to a grow out facility and sampled for leg bone measurements at D21 (N = 162) or D35 (N = 162).

On E13, femoral ossified percentage was higher for 12L:12D than for 24L (+2.8%) and 24D (+3.2%; P = 0.002) and on E14, it was higher for 12L:12D than for 24L (+5.5%; P = 0.008). At hatch, femur length was higher for 12L:12D than for 24D (+0.32 mm) and 24L (+0.45 mm; P < 0.001). Tibia weight differed among treatments (P = 0.02), but after Bonferroni adjustment, LS Means were no longer significantly different. At day 21, tibia length was higher for 12L:12D than for 24L (+1.62 mm; P = 0.01). At day 35, femur depth was higher for 24D than for 24L (+0.28 mm) and 12L:12D (+0.23 mm; P = 0.01). Femur weight was higher for 12L:12D than for 24L (+0.65 g; P = 0.03).

To conclude, applying a 12L:12D rhythm during incubation had a stimulatory effect on embryonic ossification and bone development at hatch and in the grow-out period compared to 24L in particular.

Incorrect incubation conditions can generate leg problems in poults

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The incidence of splayed legs in poults at hatch is low, normally no more than 0.50%, but it can increase notoriously on some occasions. The aetiology of the splayed legs and lameness are complex and has been associated to genetics, nutrition, infection, management, environmental and recently to incubation conditions. The ossification process in turkeys begins during the embryonic period. Simsa and Monosonego-Ornan (2007) detected signs of ossification, such as collagen type X, alkaline phosphatase, and expression of metalloproteinases at 18 d of embryo development. The highest growth rate of bones occurs a couple of days before hatch and a few days post-hatch (Ballock and O'Keefe, 2003). Therefore it is crucial that the incubation conditions be ideal to not affect bone development (Yalçin and Siegel, 2003). Temperature has been suggested to be the most important factor controlling embryo growth and development (Meijerhof, 2000). Higher temperatures during incubation can affect bone, tendon and muscle development, and thyroid metabolism (Oviedo-Rondón et al., 2008). Temperature has an important influence on the thyroid-IGF1-GH hormonal axis that controls growth plate chondrocyte differentiation, and in general bone development (Christensen et al., 2005). High temperatures also depress the expression of collagen type X and Transforming Grow Factor Beta, two important proteins involved in bone ossification. Additionally, to accelerate embryo growth to rates that demand higher oxygen consumption than can passively diffuse through the pores of the eggshell, the embryo shifts energy metabolism from lipids of the yolk, which requires oxygen, to glycogen that the embryo stored in muscles (Oviedo-Rondón and Wineland, 2011). If the yolk is not absorbed during this period, bones will not receive nutrients critical for their early development and bone modeling and remodeling. The overheated poults may have lower muscular strength to stand up at hatch because they have lower glycogen reserves in the muscles and their myofibers are also thinner (Molenaar et al., 2011). When acidity increases important contractile and metabolic functions of muscles are hindered. In the case that acidity is not regulated, the accumulation of lactic acid may be a factor in muscular fatigue (Christensen et al., 2007). Sometimes, this effect can be severe and cause late embryo mortality, but frequently the overheated poults that hatch will be lethargic, may appear exhausted, slow to search for feed and water, and potentially become the starve outs at the farm increasing the first week mortality (Oviedo-Rondón and Wineland, 2011).

It has been reported that early low and later high incubation temperature can generate thinner gastrocnemius tendon fibers and differing collagen banding patterns during subsequent growth. Christensen et al. (2007) reported that in turkeys, incubation temperatures higher than 38°C and O2 concentrations below 21% at the plateau affected muscle growth and physiology.

In conclusion ossification of bones begins during embryonic period. Independent of the turkey strain stressful conditions during the artificial incubation such as high temperature and low levels of oxygen can affect the bone development.

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Effect of locomotor activity on bone parameters, performance traits and walking ability of heavy turkey toms

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Genetic selection improved growth rate as well as feed conversion rate of modern turkey lines. On the other hand this selection for meat production has altered the relative growth of the locomotory system and the organs of the body (Nestor et al, 2005, Hünigen et al., 2012). Skeletal disorders and lameness account for considerable problems in commercial turkey husbandry. Besides genetic selection and feed restriction, an increase of locomotor activity is referred to reduce leg problems. It was found that walking exercise of young birds can positively influence the potential for running in adult birds and improved the bone stability and decreased leg disorders (Reiter and Bessei, 1998; Berk and Cottin, 2005, Rutten, 2000).
The aim of this study was to investigate the effect of locomotor activity on bone parameters, performance traits and walking ability of heavy male turkeys.

A total of 744 one day-old male turkeys of the line B.U.T. 6 were randomly allocated to 6 pens (36 m² each) holding 124 ani-mals each. Group 1 (n=248) received no special training while group 2 (n=248) was trained from 2 to 8 weeks of age and Group 3 (n=248) was trained from 2 to 21 weeks of life. Training started at two weeks of age with a running distance of 50 m. At this time birds were trained 3 times a week. From week 3 to 21 they were trained 5 times (days) per week, with an enhancement of 50 m per day every week up to 300 m in their 7th week of life. Turkeys were trained as groups based on compartments with diminishing group sizes from 124 animals (wk 2-8) to 10 birds (wk 19). The following traits were recorded: body weight, food consumption, mortality, weight and length of femora and tibiae. In the middle and at the proximal end of tibiae and femora total area, total density, cortical area, cortical density and strain strength index were recorded by peripheral quantitative computer tomography. Additional, breaking strength of bones, walking ability and leg posture were assessed.

There was no significant effect of exercise on growth, mortality or feed intake. Training reduced leg abnormalities, improved walking ability and increased total density and breaking strength of femora ($p<0.05$). In conclusion, results showed that a systematic training of fast growing male turkeys can be a way to reduce leg problems in tom turkeys without negative impact on performance traits.

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Session 8
Student´s competition and podium discussion on embryonic development of body functions and developmental reprogramming by maternal influences

Ventilation changes associated with maturation of an endothermic phenotype at hatching in Pekin duck, *Anas platyrhynchos*

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Birds begin embryonic life with an ectothermic phenotype and develop an endothermic phenotype after hatching. Precocial species, like the Pekin duck, make this transition rapidly upon hatching. Switching to a high-energy endothermic phenotype requires high functioning respiratory and cardiovascular systems to deliver sufficient oxygen from the environment and to the tissues. Here we examined tidal volume ($V_{T)}$, breathing frequency (f), minute ventilation ($\dot{V}_E$), and whole animal oxygen consumption (V_{O2}) during the developmental transition from an ectothermic externally pipped (EP) paranate to an endothermic hatchling.

We measured V_{O2}, V_T, and f as animals gradually cooled from 37°C (EP) or 35°C (hatchling) to 20°C. An additional set of experiments examined hatchling responses to increased CO_2. The barometric technique was used to estimate V_T. Only hatchling V_{O2} significantly increased in response to cooling. Paranates had a high f that decreased with cooling, whereas hatchling f was significantly lower and increased with cooling. Hatchling V_T was significantly higher compared with that of EP paranates. During cooling, V_T increased only in hatchlings. Hatchling ($\dot{V}_E$) increased significantly during cooling, mainly due to increased f, whereas paranate ($\dot{V}_E$) remained constant. Increasing CO_2 to 4% resulted in a significant increase in hatchling f, V_T, and ($\dot{V}_E$).

We suggest that an endothermic ventilator response of EP paranates is constrained by the rigid eggshell, limiting expansion of the air sacs during inhalation and constraining V_T. Upon hatching, this V_T limitation is removed and the animal is able to increase V_T and ($\dot{V}_E$), and thus V_{O2} and exhibit an endothermic phenotype.

How to Build a Furnace: The Role of T3 in Development of Endothermy in Altricial Birds, including the Red-winged Blackbird (*Agelaius phoeniceus*)

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Avian development occurs across a spectrum of functional maturity at hatching ranging from precocial to altricial. Altricial red-winged blackbirds (RWBB) rapidly mature in the nest before fledging around 11 days post hatch. During this period, neonates undergo physiological and metabolic changes associated with development of endothermic phenotype.

Thyroid hormones (TH), key regulators of avian metabolism, are thought to influence development of endothermy. Altricial species, which remain non endothermic for days after hatch, show similarly delayed maturation of the thyroidal axis. This delayed timing suggests a relationship between peak circulating TH and obtainment of endothermy.
To better understand the role of TH in RWBB endothermic developmental trajectory, we characterized systemic O_2 consumption (VO_2) and ventilation, and mitochondrial respiration of permeabilized fibers from breast, thigh, and heart after plasma TH levels were altered via administration of the thryoperoxidase inhibitor, Methimazole (MMI), during the first 5 days of neonatal life.

5 days post-hatch (dph) MMI treated animals exhibited lower plasma T_3 than control. Body mass of hypothyroid animals did not differ from control, but fractional heart mass of 5 and 7dph was lower. Hypothyroid animals showed delayed maturation in VO_2 response when faced with decreasing temperature, but achieved an endothermic phenotype by 9 dph. Wing chord and femur length of MMI hatchlings was shorter than control. Hypothyroid neonates had lower mitochondrial respiration when compared with control animals.

Our data suggest TH plays an active role in systemic development of endothermic capacity, especially in the first days after hatching for fast growing species like the RWBB. In the neonate avian multiple systems develop in concert to produce an endothermic phenotype, but reduced TH can delay maturation of endothermic capacity in altricial species.

Embryonic development and heat production of embryos from two modern broiler strains

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Modern broiler strains are intensively selected for high growth rate at a low feed conversion ratio. These production traits might have an influence on embryonic development and heat production during incubation.

To examine the effects of broiler strains, hatching eggs of Ross 308 and Cobb 500 fast feathering were selected from breeder flocks aged 43 to 46 weeks at an egg weight range of 60 to 63 g. Eggs were obtained in 2 batches, 120 eggs per strain per batch. For each batch, 20 eggs per strain were used to determine egg composition. The remaining eggs were incubated separately in 1 of 2 climate respiration chambers at an eggshell temperature of 37.8 ◦C.

The results showed that Ross 308 eggs had a higher yolk: albumen ratio with 0.9 g more yolk and 0.7 g less albumen than Cobb 500. Cobb 500 and Ross 308 embryos had similar growth rate during the first two weeks of incubation. At incubation day (E) 18, Ross 308 embryos tended to have a heavier yolk free body mass (YFBM) than Cobb 500 embryos. At hatch Ross 308 chicks were 0.2 cm longer and had a 0.6 g heavier YFBM than Cobb 500 chicks. Absolute and relative heart and liver weights did not differ between strains. At 3 h after hatch the residual yolk of Ross 308 chicks tended to be lower than that of Cobb chicks, which suggested that Ross embryos used more yolk during incubation. Egg weight loss at E18 tended to be higher in Ross 308 than in Cobb 500. The moment of internal pipping did not differ; but the moment of external pipping and hatching moment was about 4 h earlier in Cobb 500 than in Ross 308. The embryonic heat production of Ross 308 was numerically higher than Cobb 500 only between E16 to E18, about 3 mW/ egg.

It can be concluded that, Cobb 500 and Ross 308 differ in egg compositions and have different trajectories for embryonic development during incubation even when egg weight and breeder age is the same.

Comparison of the hormone and nutrient yolk profile of broiler and layer breeds

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Maternally-derived nutritional and endocrine factors have been intensively studied for their developmental programming effects on offspring morphology, physiology and behaviour. Recent studies indicate a complex, synergistic response of breed-specific genotype and yolk environment initiates very early on in embryonic development (Ho et al., 2011). Such 'developmental reprogramming' events have been associated with changes in yolk hormone and nutrient concentrations. In light of this phenomenon, extensive analysis of compositional changes in yolk profile of two distinct commercial breeds was studied prior to incubation to identify potential maternally-derived epigenetic mediators.

200 fertile eggs from each of Cobb 500 Broilers and White Leghorn Layers were measured for total egg, yolk and albumen weight. Individual yolk samples within each breed were pooled based on stratified egg weight and analysed for dry matter, lipid, protein, glucose and developmental hormones testosterone (TE), corticosterone (CORT), insulin, total triiodothyronine (T_3) and thyroxine (T_4).

Broiler eggs were significantly heavier than layer eggs (60.19 ± 0.29 g vs. 57.58 ± 0.29 g, $P<0.001$) associated with increased relative yolk weight (16.61 ± 0.07 g vs. 12.97 ± 0.07 g, $P<0.001$). Layer yolk contained significantly higher concentrations of glucose than broiler yolk (1.93 ± 0.07 mg g-1 vs. 1.64 ± 0.07 mg g-1 $P<0.05$), and while not significant, protein concentration displayed a similar trend ($P=0.16$). Layer yolk expressed significantly higher insulin concentrations than broiler yolk (1.28 ± 0.032 pg mg-1 vs. 1.03 ± 0.031 pg mg-1 $P<0.001$). The increased yolk glucose and protein concentrations in layer yolk may reflect breed-specific feeding management practices, as layer hens are fed ad libitum while broiler breeders are generally feed restricted.
Broiler yolk contained significantly higher TE concentration than layer yolk (0.64 ± 0.03 pg mg-1 vs. 0.55 ± 0.03 pg mg-1, $P<0.05$) accompanied by a two-fold increase in T_4 (4.52 ± 0.12 pg mg-1 vs. 2.36 ± 0.13 pg mg-1 $P<0.001$). These results are supportive of the increased growth and development rate previously observed in broiler embryos.

In conclusion, differences in yolk concentrations of nutrients (glucose, protein) and hormones (insulin, thyroxine, testosterone, corticosterone) exist prior to onset of embryonic development. However, characterising genetic differences is difficult as yolk concentrations reflect levels found in hen circulation, which in turn is influenced by breed-specific genotype and the maternal environment. It is likely differences in hormone concentrations play a critical role in epigenetic mediation of broiler and layer embryonic development and phenotypic expression however further studies disseminating individual genetic and maternal environmental, particularly feed restriction, effects on yolk deposition is required to gain a better understanding of the role yolk insulin, glucose, TE, and T_4 play in embryonic reprogramming and the underlying mechanisms.

HO, D. H., REED, W. L. & BURGGREN, W. W. 2011. Egg yolk environment differentially influences physiological and morphological development of broiler and layer chicken embryos. J Exp Biol, 214, 619-28.

Reprogramming of Broiler Growth and Immunity through Changes in Breeder Hen Bodyweight

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Reprogramming of developmental events is increasingly recognised as having lifelong effects on animal health, welfare and productivity. Whilst most studies have concentrated on metabolic effects of reprogramming, the immunity of the progeny may also be compromised. This is of particular significance in broiler birds which are prone to infectious diseases post hatch. Broiler breeder hens are feed restricted to 50% of ad libitum feed intake, leading to a state of chronic hunger. This persistent hunger may cause stress to hens, leading to re-programming of progeny immunocompetence. This study examined the link between maternal stress caused by feed restriction in hens and the ability of their offspring to respond to an immune challenge.

Thirty six Cobb 500 broiler breeder hens were maintained at three levels of bodyweight; 3.4kg, 3.6kg and 4.0kg, over 19 weeks. Hen behaviour was observed daily using an ethogram over two weeks of lay, and serum was collected at 31 weeks for corticosterone levels. From these hens, 170 viable chicks were hatched, weighed and placed into group rearing pens of ten birds from the same hen treatment group, with three replicates of each group. Half of the chicks from each hen were given a series of three injections of lipopolysaccharide (LPS) E.coli O55:B5 at 16, 18 and 20 days old. Birds were injected at a dose rate of 0.5 mg/kg bodyweight, intraperitoneally. Blood samples were collected from the brachial vein of three birds per pen on days 21 and 35 and heterophil/lymphocyte (H/L) cells were counted. H/L counts were completed by counting one hundred cells per slide, three times. Birds were grown until 42 days old.

Hens maintained at a lower bodyweight showed increased pecking behaviour compared to hens at a higher bodyweight ($P<0.05$). Corticosterone levels were also higher in low bodyweight hens ($P=.013$). Together these results indicate an unfulfilled hunger drive and possible stress in these birds. Hen bodyweight also influenced progeny growth from days 35 to 42 in male birds ($P<0.05$). Males from heavy hens grew heavier in this week than those from medium and low bodyweight hens. Sex effects were also observed on day 23 H/L counts ($P<0.05$) with a higher H/L ratio in female progeny from heavy hens compared to male birds from all hens, and females from low and medium bodyweight hens, demonstrating an effect of hen bodyweight on the response of female birds to an LPS challenge. Females from heavy hens were therefore more sensitive to the LPS immune challenge and increased immune cell numbers to a greater extent than those from hens restricted to lower bodyweights.

From this study, a link between hen bodyweight and progeny growth and immunity was demonstrated through differences in growth, circulating immune cell counts and response to an immune challenge (LPS).The mechanism behind these differences needs to be investigated further as well as differences between males and females observed in this study.

Session 9
Endocrine disruption

Keynote: Phenotypic changes by endocrine disruptors - amphibians as model organisms to assess their modes of action

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Endocrine disruption by various compounds has become an emerging concern especially for aquatic wildlife because surface waters are the main sink of endocrine disruptors (ED). Amphibians are the classical model organisms to assess the modes of action of ED because it has been shown that all phenotypic changes associated with metamorphosis from tadpoles to juveniles are triggered by the thyroid system and the bioavailability of thyroid hormones. In addition, first classical experiments in the 50`s of last century concerning phenotypic sex reversal demonstrated that the sex steroids, estrogens and androgens, can lead to feminization and masculinization due to changes of the hormonal relation between estrogens and androgens during larval development. In the past ED research focused on (anti)estrogenic, (anti)androgenic, and later on also on (anti)thyroidal substances affecting reproduction and development in vertebrates but further endocrine systems might be also targets for ED. In the model organism *Xenopus laevis (*South African clawed toad) impacts of (anti)estrogenic and (anti)androgenic ED affect sexual differentiation during larval development but also during adult stages affecting the hypothalamus- pituitary-gonad (HPG)-axis and induces also drastic behavioural changes concerning male mating calls. (Anti)thyroidal ED affect the bioavailability of thyroid hormones in general or in determined tissues and thus induce drastic phenotypic changes associated with metamorphosis and development by impacts on the hypothalamus-pituitary-thyroid (HPT)- axis or direct effects on thyroid or on deiodinases differentially expressed in various tissues. More recently environmental gestagens, including the natural progestogens, e.g. progesterone, and synthetic progestins, have been identified as potential ED. Gestagens have been supposed to affect vertebrate reproduction via progesterone receptors especially by progestins being the major compound of the “mini pill” for contraception of humans and thus progestins are also present in surface waters. Amphibians are suitable models to assess endocrine disruption and also targets of gestagens. Exposure to progestogens such as progesterone seems to be less effective in comparison to progestins. During larval exposure the progestin levonorgestrel (LEVO) disrupts sexual development in *Xenopus laevis* by affecting gene expression of pituitary gonadotropins and gonadal steroidogenic enzymes. In Xenopus tropicalis larval exposure to LEVO had long lasting effects on adults affecting especially females lacking oviducts and having histopathological patterns of the ovaries. Surprisingly, in parallel LEVO also impairs metamorphosis by disruption of the thyroid system. However, the underlying molecular mechanisms need still to become elucidated. Recently, in order to get a better insight into the mechanisms how ED e.g. gestagens affect phenotypes a shift from in vivo experiments to in vitro organ cultures of thyroid and gonads is our strategy to reveal potential direct effects of ED on target organs.

Short-term prenatally elevated glucose level acts as metabolic disruptor on hypothalamic neurons regulating body weight and metabolism – a lesson from the chicken

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Early life environmental experiences may have a distinct impact for long-term health. Exposure to maternal 'diabesity' during pregnancy increases offspring 'diabesity' risk, e.g. by malprogramming the central nervous regulation of body weight, food intake and metabolism. Critical mechanisms and concrete disrupting factors still remain unclear. Due to the independent development, from the mother, the chicken embryo provides an excellent model to distinctively establish causal factors. As 'closed system' it allows to examine and thereby to identify concrete risk factors under well-controlled and highly standardized conditions. Further, similar to mammals also in birds feed intake and body weight are regulated via neuronal circuits located in the hypothalamus. Therefore, we aimed to investigate consequences of short-term exposure to high glucose (hyperglycemia) within a well-defined critical period of prenatal development on postnatal hypothalamic neuro-metabolic function in the chicken model.

To temporarily induce high-glucose exposure in chicken embryos, 0.5 ml glucose solution (30 mmol/l) were administered daily via catheter into a vessel of the chorioallantoic egg membrane from days 14 to 17 of incubation. At three weeks of postnatal age, body weight, total body fat, blood glucose, neuronal hypothalamic glucose sensitivity, mRNA expression of Insulin receptor, leptin receptor as well as glucose transporters (GLUT1, GLUT3) as well as corresponding promoter DNA methylation were determined in mediobasal hypothalamic brain slices (*Nucleus infundibuli hypothalami)*. The results were compared with data from NaCl-treated control group and, additional, for neuronal glucose sensitivity with data from a non-treated control group.

Although no significant changes in peripheral metabolic parameters were found, high hypothalamic glucose-resistance und strongly decreased mRNA expression occurred in all candidate genes due to temporarily experiences of prenatal hyperglycemia during the 'critical period' of the HAP-development. However, no relevant alterations were observed in respective promoter methylation.

In conclusion, data indicate in a translational sense that elevated glucose acts as a 'metabolic disruptor' during central nervous development, leading to a persistent malprogramming and 'set-point' alteration of its own central nervous functioning at the cellular and expression level of candidate genes addressed here. Acquired neuronal and molecular alterations might contribute to increased 'diabesity' risk throughout life. At the same time, absence of related changes in promoter DNA methylation seems to challenge an over-simplified favoring of respective promoter epigenomics as the principal mechanism in prenatal imprinting and central nervous programming.

Session 10
Maternal and paternal effects, nutrients and hormones

Hormone mediated maternal effects and the role of the embryo

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In many animal species embryos are exposed to hormones of maternal origin, affecting their development and final phenotype. Birds are excellent models to study this as the embryo develops outside the mother's body in relatively large eggs, facilitating measurements and manipulation of hormone exposure without interfering with the mother. In addition, bird eggs contain substantial amounts of these hormones, showing systematical variation within and among clutches and between species. Moreover, hormone deposition is depending on environmental factors.

The prevailing hypothesis is that mothers may strategically adjust hormone concentrations in the egg to increase her fitness. However, her fitness may not always correspond with that of the offspring, leading to a parent-offspring conflict. Moreover, maternal hormones can only be effective if the embryo can take up and use these hormones in the right developmental phases.

We therefore study the role of the embryo in in taking up, and conversion of these hormones in the yolk and in the brain. We found that concentrations of maternal steroids in the egg are highly dynamic, is converted to several other hormones, and that conversion in the brain can be related to the position of the egg in the clutch, determining the chick's position in the sibling hierarchy.

Selection for contrasting yolk testosterone deposition affects HPG axis of male Japanese

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Androgens deposited in the eggs by the avian mother may programme physiological and a behavioural phenotype of offspring. Maternal testosterone (mT) deposition is related to a genetic background of the mother and reflects environmental and social conditions during egg formation. Since mT is deposited by mothers it is expected that its content in the egg yolk is exclusively determined by the female. However, since our results experimentally proved high heritability of this trait it is possible that maternal T deposition is to some extent affected also by the male.

We tried to answer this question on the basis of results obtained from our bidirectional selection of Japanese quail for low (LET) and high (HET) egg testosterone content. Selection resulted in establishing two strains and the HET line exceeded twice LET line in content. Breeding of females from both lines with males from control random bred population resulted in an increase in egg T in LET and the decrease in the HET lines.
Basal plasma LH and T levels did not differ between LET and HET males but the response of LH to GnRH was higher in HET than LET males.

Our results suggest that selection for high egg T deposition increased sensitivity of the hypothalamo-pituitary axis in male Japanese quail. Genetic background of males can therefore influence deposition of maternal T in their daughters with possible consequences for their progeny performance.

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Egg deposition of maternal testosterone is primarily controlled by the preovulatory peak of luteinizing hormone

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Variability of androgen concentrations in avian eggs is often explained by an adaptive hypothesis according to which differential maternal deposition of yolk hormones may adjust offspring's phenotype to ambient environmental conditions. In line with this hypothesis, numerous studies have shown that experimentally increased yolk testosterone levels affected a wide array of offspring's traits. However, a mechanistic view on the variability of yolk androgen deposition is still missing. To understand physiological mechanisms of egg hormone deposition, we analysed a temporal pattern of plasma luteinizing hormone (LH), testosterone and estradiol concentrations during the ovulation-oviposition cycle in two lines of Japanese quail that were divergently selected for low (LET line) and high (HET line) yolk testosterone levels.

After six generations of selection, HET females laid eggs with more than twice yolk testosterone concentrations as LET females. Exact time of egg laying was recorded for each female over one week-period to estimate timing of individual ovulation-oviposition cycle and then serial blood samples were collected at 6.5, 3.5 and 0.5 hours before expected ovulation. In the second experiment, we evaluated responsiveness of LH to a single stimulation with an analogue of gonadotropin releasing hormone (GnRH) in females of both lines The GnRH challenge was performed around 3.5 hours before ovulation. In HET females, the highest LH levels were found 3.5 hours before ovulation and they corresponded to the expected preovulatory LH peak. Surprisingly, in LET females, maximum LH concentrations were reached 0.5 hours before ovulation. Moreover, plasma LH levels were significantly higher in HET than LET females 6.5 and 3.5 hours before ovulation with no line differences around the time of expected ovulation. Preovulatory peaks of plasma testosterone and estradiol concentrations were found between 6.5 and 3.5 hours before ovulation in both LET and HET females. Plasma LH levels increased five minutes after direct GnRH stimulation but the responsiveness did not differ between lines.

In conclusion, our results demonstrated that high yolk testosterone deposition is associated with the preovulatory peak of LH in the circulation and probably depends on factors that influence hypothalamic-pituitary sensitivity during the ovulation-oviposition cycle.

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Effects of *in-ovo* thyroxin injection with or without arginine feed supplementation on hatchability, performance and cold tolerance acquisition in broilers

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This experiment was conducted to evaluate the effects of thyroxin via *in--ovo* injection with or without arginine feed supplementation on hatchability, post hatch performance and cold tolerance acquisition in progeny chicks.

A total of 2400 hatchings eggs were randomly assigned to four treatments (8 replicates for each treatment and 75 eggs for each replicate). Treatments were: 1 & 2) control (intact or pricked with a needle), 3) injected with distilled water and 4) injected with thyroxine. On the hatching day, 240 one-day old male broiler chicks from the intact and injected with thyroxine treatment groups were reared for 42 d with or without dietary arginine supplementation in a 2×2 factorial trial based on completely randomized design where the injection conditions (injection or non-injection) and the arginine feed supplementation (with or without) were considered as the factors. In order to induce ascites in chicks, all of the chicks were exposed to 15°C from 15 to 42 d of age.

The results showed that the second grade chicks and yolk sac weight were decreased ($P<0.05$) in thyroxine via *in-ovo* injection. Final body weight and feed conversion ratio were not affected by experimental treatments. Ascites mortality rate was decreased ($P<0.05$) by thyroxine injection and arginine supplementation. The mean of packed cell volume percent, red blood cell count were significantly decreased ($P<0.05$) by *in-ovo* injection of thyroxin and dietary arginine supplementation.

In conclusion, these results show that *in-ovo* injection of thyroxin and dietary arginine supplementation improves the performance during exposure to cold environments.

Early phenotypic and metabolic characterizations of two chicken lines divergently selected on their breast meat ultimate pH

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In chicken, several studies have shown that the meat ultimate pH (pHu) is largely defined by the glycogen content in the muscle at slaughter. Genetic analyses have shown that the muscle pHu is highly heritable in chicken. On this basis, a divergent experimental selection on the breast meat pHu has been initiated. The pHu- and pHu + chicken lines were obtained, which represent a unique model to study the genetic and physiological control of glycogen-related meat quality defects. These lines have recently been characterized at slaughter age. They exhibited after 5 generations of selection 0.5 pH-unit difference in the breast muscle that is associated with large differences in muscle glycogen and meat quality traits (color, water-holding capacity and texture). The pHu+ line also exhibited higher breast and leg meat yields than the pHu- line. The purpose of our study was to characterize the pHu+ and pHu- lines at hatch and evaluate the consequence of the post-natal nutrition (5 days) on their early metabolism and growth performances.

Several traits were measured including body weight, muscle yields and pHu, plasmatic parameters (glycaemia, triglycerides, uric acid, free amino acids), and activation by phosphorylation of several signaling pathways involved in both protein synthesis and glycogen turnover (P70S6K/S6, AMPK/GYS and GSK3/GYS).

At hatch (before any nutrient supply), the pHu+ and pHu- lines exhibited similar body weight, muscle yield and pHu. However, the pHu- line showed higher glycaemia than pHu+, a difference that persisted until slaughter age. Primary muscle cell culture of hatched-chicks revealed different capacities to respond to insulin between lines. After 5 days, the pHu+ and pHu- lines were already divergent for breast muscle pHu (0.4 pH-units) and yield for a similar body weight. The differences observed at this age were associated with changes in phosphorylation level of different signaling pathways involved in energy and protein metabolism.

In conclusion, chickens from the pHu+ and pHu- lines presented at hatch different capacities to respond to nutrients and hormones that certainly explain why only 5 days of food supply are sufficient to induce muscle yield and pHu differences between lines.

Session 11:
Epigenetic modification and stress

Short-term temperature training in the hatcher improves stress response in broiler chickens? – First results from behavioural observations and blood analysis

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An alternative approach to improve functional traits alongside with the production efficiency of fast growing broiler chicken lines provides temperature training in the hatcher (PTT: perinatal temperature training from day 18 until hatching) with short-term mild warm loads. Temperature training of the developing thermoregulatory system during critical periods has long lasting effects on thermal adaptability and various body functions, because of the strong relationship between the central control of body temperature and body functions, like metabolism, feed intake and body weight regulation as well as immune und stress response. The hypothesis is that perinatal temperature training improves robustness via long-lasting reduction of the basic metabolism. In previous experiments we found especially in male chickens a lower hypothalamic neuropeptide-Y (NPY) expression as long-lasting effect on basic metabolism after PTT. Hence, robust chickens have more energy available for adaptation, immune and stress responses during environmental challenges.
In two pilot studies (summer 2012, spring 2013) the influence of short-term perinatal temperature training on stress level and welfare in broiler chickens (Ross 308) was investigated. Eggs were incubated under commercial conditions using incubators with total capacity of 115.200 eggs (SmartSet □, Pas Re
The eggs were incubated under standard single stage incubation programme (control) or with PTT in the hatcher (+ 1°C, maximum 2 hrs per day). Random sampling (120 males and 120 females) of hatched chickens from control and PTT group was used for subsequent broiler growth trial of 35 days in the experimental research station of the FLI (Federal Research Institute for Animal Health, Institute for Animal Welfare and Husbandry in Celle, Germany). During the growing period locomotor activity was observed. On day 34 fear response was examined using a novel object test (NOT). Blood samples for hormone analysis (T3/4, cortisol, corticosterone) and preparation of blood smears for calculation of heterophile to lymphocyte ratio (HLR) were collected. Locomotor activity was not different between the groups. However, it must be pointed out that the chickens in the PTT group have a higher body weight compared with the control. In the NOT a slight tendency to less fear response was found. HLR was statistical significant lower in the PTT group than in the control group. Acute stress (e.g. during slaughtering) is typically related to increase in energy mobilization. Hence, our hypothesis was that PTT chickens, especially the males, can mobilize more energy during acute stress. This hypothesis was confirmed. Male chickens have higher increase in blood T3/T4 level during acute stress, which was accompanied by similar increase in stress hormone level (cortisol and corticosterone). In females only slight changes in metabolic and stress hormones were observed, which corresponds with NPY expression in a previous experiment. It has to be noted that all results are similar in both growing trials. It means that the long-lasting effect of PTT was repeatable.

Epigenetic modifications affect the expression of corticotrophin-releasing hormone (CRH) which is involved in the mechanism underlying the balance between heat stress resilience and vulnerability

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Determining whether heat exposure will lead to future heat-resilience or vulnerability depends on a delicate balance of a properly adjustable heat response set-point. The adjustment of this set-point is most likely effective during sensory postnatal development and involves the hypothalamus-pituitary-adrenal (HPA) axis. Here we demonstrate that heat stress during the critical period of thermal control establishment in 3-day-old chicks, renders habituated or sensitized response, a week later, depending on the ambient temperature i.e. moderate heat leads to future heat resistance while harsh temperatures lead to heat vulnerability. Furthermore, these changes might be governed by epigenetic modifications, specifically DNA methylation.

The mRNA expression of CRH in the hypothalamic paraventricular nucleus and plasma corticosterone were elevated a week after heat conditioning in chicks which were trained to be vulnerable to heat, while it declined in chicks that were trained to be resilient, demonstrating correlative changes in the HPA axis.

Interestingly, the DNA methylation pattern along the CRH gene changed significantly between the groups, a week after their heat conditioning. In order to study the role of plasticity in the HPA axis, CRH or antisense to CRH were intracranialy injected into the third ventricle. CRH caused an elevation both in body temperature and plasma cort level, while CRH-antisense caused an opposite response. These opposite responses were memorized a week later. This effect was used to reverse resilience into vulnerability and vice versa. Chicks that have been injected with CRH followed by exposure to mild heat stress, normally inducing resilience, demonstrated a very high elevation in body temperature and CRH expression while chicks that were injected with CRH-antisense and exposed to harsh ambient temperature were reversed and responded instead of the expected vulnerability in long-term heat resilience.

These results demonstrate a role for CRH in determining either heat resilience or vulnerability response later in life.

POSTERS

Embryonic development, environmental and maternal effects

1 - Stages of embryonic development in chickens

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This study aimed to characterize the stages of embryonic development in broilers, for educational and scientific purposes using photographic methods.

330 eggs from Cobb® broiler breeder flock (45 weeks old) were collected at the farm on the same day and after the first general collection, to avoid the use of eggs laid on the previous day. The incubation procedure followed the routine of the industrial hatchery, and the eggs were submitted to three days of storage under average conditions of temperature and relative humidity of 19.5°C (67°F) and 82 %, respectively. After this period, 10 hours of pre-heating occurred under average conditions of temperature and relative humidity of 28.2°C (83°F) and 76 %, respectively. Subsequently, the eggs were incubated in a Casp® CMg 125E multistage machine with a capacity of 124,416 eggs. The incubator had its thermostat set to maintain a constant temperature at 37.4°C (99.3°F). Automatic turning occurred every hour and after 19 days of incubation the eggs were transferred to the Casp® G21E hatcher with a capacity of 20,736 eggs. In this machine the thermostat was set to maintain an average temperature of 36.6°C (98°F). Every 24 hours, a group of 15 eggs was removed from the machine. These eggs were broken and placed in Petri dishes to obtain the photographic images. The photos were taken by D-SLR Nikon® camera and AF-S 105mm close-up lens. After the fifth day of incubation, the eggshell was carefully removed with the aid of anatomical dissection tweezers to preserve the structures.

It was possible to identify the stages in the development of the chicken embryo, described by Hamburguer and Hamilton (1951), and demonstrate the organ differentiation, the internalization of the yolk sac into the abdominal cavity and important moments during the incubation, such as internal pipping, external pipping and hatch.

2 - Investigating the role of amniotic and chorioallantoic fluids in the protection of the chicken embryo

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Chicken egg is initially a germ-free chamber, which contains all the nutrients and biological activities required to support the development of an embryo and its protection against microbial attacks. These protective systems are of major importance to prevent bacterial contamination of embryonic eggs and to ensure safety of unfertilized eggs (table eggs) for consumers. To date, more than one hundred different proteins have been identified as potentially involved in the defense of unfertilized eggs. However, little is known about the regulation of these defenses during embryonic development. Indeed, the major defenses, which are initially present, are rapidly modified even altered during incubation. Eggshell internal components which form a physical barrier against microorganisms are dissolved for the calcium requirements of the embryo. This partial disintegration is likely to be associated with a weakening of the eggshell and its protective role. On the other hand, the yolk and the egg white which contain maternal antibody and antimicrobial molecules, respectively, are gradually absorbed by the embryo during its development. Therefore, it is hypothesized that alternative systems could develop to protect the embryo. To address this question, we propose to characterize components of two extra-embryonic fluids that are progressively formed during development: amniotic and chorioallantoic fluids.

Indeed, while yolk and albumen disappear during incubation, the two extra-embryonic fluids expand through the egg, surrounding the embryo, and may act as a protective barrier. The amniotic fluid participates in the protection of the embryo against mechanical shocks and is mixed with the egg white from the 12th day of the development onwards, before its oral absorption by the embryo. The chorioallantoic fluid is the product of the urinary system and provides water for the embryo in the second part of its development. The protein compositions of these two fluids have never been characterized. We first analyzed the fluids at various stages of incubation (from day 8 to day 16) using biochemical methods and measurements of their physicochemical properties, to select stages of interest. The next step will use proteomics and nuclear magnetic resonance to follow evolution of the fluids components including antimicrobials during incubation and to identify potential new antimicrobial molecules. In parallel, we will explore the antimicrobial potential of the fluids using antibacterial assays.

Altogether, these data will help to better define the role of these fluids in the protection of the embryo during its development to prevent bacterial contamination. Moreover, we expect to identify new antimicrobial components that could be further purified and characterized and that might be of interest as alternatives to antibiotics.

3 - Development of endocrine pancreas of the chick embryo

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We described development of pancreas in the chick embryo with the aim to characterize growth and differentiation of its endocrine compartment.

Pancreas originates in the chick embryo as three outgrowths of the dorsal wall of duodenum during 2 ½ - 3 days of embryonic development (d). They form three lobes (ventral, dorsal and splenic one) consisting of exocrine cells organized in tubules and endocrine cells located near their epithelium. It is possible to find individual endocrine cells as well as their groups and whole Langerhans' islets. Among them are distinguished „light islets" consisting largely of cells B producing insulin and big „dark islets" composed of cells producing glucagon and somatostatin. The hormone production was detected mainly from day 3 (first glucagon, later insulin and somatostatin, and later also pancreatic polypeptide). The ratio of exocrine to endocrine tissues first prevailed in favor of endocrine ones. It changed after day 9, when a rapid growth of exocrine tissue began. Our investigation of proliferation by FACS showed that at stages 9,12,14 and 16d regularly 10% of cells in the pancreas anlage occur in S and G2 phase. The only exception was found at day 11, when the proliferation increased to 24%, probably thanks to growth of exocrine cells.

The immunohistochemical investigation of pancreas in 5-, 7-, 9-, 12-, 16-, and 18-day old embryos confirmed early functioning of endocrine tissue: we demonstrated insulin-, glucagon- and somatostatin-immunoreactive cells already from day 5. Using immunohistochemical reaction we accentuated the presence of chondroitin-sulphate proteoglycan for demonstration of basal membranes and connective tissue around the epithelia.

To assess the ratio between specialized cells in growing endocrine tissue, we measured volume density of insulin-, glucagon- and somatostatin--immunoreactive cells using morphometric analysis: an estimation of volume density of various components on random sections by measuring relative areas of their profiles, also called areal density of the profiles on section using the Delesse's principle. Ratios were calculated between particular components. The volume density of endocrine cells and their ratio appeared stable in individual lobes but varied significantly among them. Increase of the glucagon volume density was exponential, whereas insulin volume density increased almost linearly, especially in the splenic lobe. Growth of endocrine tissue resulted in the predominance of the hormone-immunoreactive cell volume density of glucagon--immunoreactive cells, which is typical for birds.

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4 - Effect of acute hypoxia on the mechanical and electrical properties of the isolated skeletal muscles in the last third of chick embryogenesis

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Cyclic embryonic motility is involved in the complex response of the chick embryo to acute hypoxia, the changes in function of skeletal muscles not being well known. The aim of our study was to describe the age-related changes of the contractile and electrical responses of fast (m. tibialis anterior) and slow (m. soleus) isolated skeletal muscles and estimate their changes under acute hypoxia in the last third of chick incubation (days 16-20).

The amplitude-temporal parameters of the single and tetanic responses of muscle as well as extracellular action potentials (APs) of the single muscle fibers (loose patch method) were investigated at different stages of incubation in normoxia, and hypoxia when the oxygen concentration in the solution was decreased three times. It was shown that the normalized values of the force of the single and tetanic contractile responses in m. tibialis anterior significantly exceeded the values of m. soleus. On incubation days 16-17, some slowly decaying oscillatory excitation waves were registered in the muscle fibers of m. soleus and m. tibialis anterior, and extracellular APs were registered in 20 % of the investigated muscle fibers in m. tibialis anterior. On the incubation day 20, the amount of muscle fibers able to generate the conducting AP was about 100 % in fast muscles while in slow fibers it was about 50 %. No significant difference in the amplitude-temporal parameters of AP between m.soleus and m.tibialis anterior was observed. On the basis of these results we conclude that it is the fast muscles that are involved in the chicken embryonic motility during the period of incubation days 16-20.

Hypoxia caused the decrease in the force of the muscle contractile responses at all ages. Moreover, under hypoxia, the index m/M characterizing the ratio of the number of fibers capable of generating AP (m) to the total number of tested fibers (M) in accordance with the law "all or nothing" was decreased almost two-fold. To understand the mechanisms underlying the changes in the muscle contractile response to hypoxia we studied the influence of caffeine, insulin and ouabain. In muscles treated with insulin and ouabain, the sensitivity of both electrical and contractile responses to hypoxia significantly decreased. At the same time, hypoxia did not impact the value of the contracture response caused by caffeine.

Consequently, we can conclude that in our investigation, hypoxia did not affect the functional state of the ryanodine receptors, and the effect of hypoxic exposure involved the active participation of membrane Na+/K+-ATPase.

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5 - Thermal stimulation improves Nitric Oxide Synthase (NOS) activity of anterior hypothalamic neurons in chicken embryos – a comparative study

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NO is one of the major factors participating in evolution of structure and function of the central nervous system. It plays, for instance, an important role in growth of nerve terminations and formation of synaptic contacts. NO is produced by activation of nitric oxide synthase (NOS). The marker for NOS-positive neurons is nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d). In our experiments on Muscovy duck embryos, an anseriform species with an incubation time of 34 days, short-term cold-stimulation induced neuronal NOS expression at earlier embryonic age (E20) and increased the number of NOS-positive neurons until hatching (E23, 28 and 33) in comparison to the control group without thermal stimulation and the warm stimulated group (DUNAI, V., TZSCHENTKE, B., 2012, The Scientific World Journal, Neurosci. doi: 1100/2012/416936). The aim of the actual study was to prove if the stimulating effect of short-term cold challenge on hypothalamic neuronal NOS activity is a fundamental characteristic of prenatal development of the neuronal NOergic system in precocial bird species. Hence, the investigations were carried out in a galliform species, the chicken embryo.

Investigations were conducted in chicken embryos on E12, E14, E18, E20 (similar age relative to the experiments in Muscovy ducklings) incubated under normal temperature (37.5°C) until the day of experiments. As in the investigations in ducks, three experimental series were performed; without acute temperature stimulation (control), with 3 hrs warm (39°C) or cold stimulation (34°C) on the respective experimental day. In the temperature-stimulated groups the brains were immediately extracted after the 3 h of temperature influence. Activation of neuronal NOS was investigated in all experimental groups using histochemistry for identification of the NADPH-d. For analysis, NADPH-d positive neurons were counted in a defined area (1000 µm x 1000 µm, subdivided into 100 squares of 100 µm x 100 µm) near the third ventricle and the *nucleus anterior medialis hypothalami*.

In the control and warm stimulated group NADPH-d positive neurons could be first detected on E14 in chicken embryos. As in Muscovy duck embryos, in chicken embryos short-term cold load induced neuronal NOS expression at an earlier embryonic age (E12). Similar to the investigations in duck embryos, the expression of hypothalamic neuronal NOS was significantly increased after cold stimulation during further development until hatching (E14, 18 and 20).

In conclusion, cold-stimulation during incubation improves neuronal hypothalamic NOS expression in both, anseriform and galliform precocial bird species. It leads to the hypothesis that possibly in precocial bird embryos NO is a common modulator of the neuronal cold and energy uptake pathway in the anterior hypothalamus.

6 - Hormone-mediated maternal effects: does the embryo plays a role in family conflicts?

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In vertebrates, the exposure of the developing embryo to maternal steroid hormones can have long-lasting organizing effects on the development of embryonic gonads and brain; as well as on post-natal morphology, physiology and behaviour. Such hormone-mediated maternal effects have been postulated as a potential tool for mothers to adjust offspring's development to prevailing environmental conditions, and thus to enhance offspring's adaptive phenotypic plasticity, enabling evolution of local adaptations. Most studies in this field have been performed with bird species where maternal steroids deposited in the egg yolk show clear and systematic variation among species, females of the same species, nests of the same mother, and eggs of the same nest.

The level of maternal steroids in yolk at oviposition is assumed to be a context-dependent maternal signal to adjust the offspring phenotype. However, the mechanisms underlying hormone-mediated maternal effects are largely unknown. Studies on bird species found a decline in free steroids over egg incubation, with an increase in supposedly conjugated forms (which are presumably biologically inactive), with one study owing this to embryonic metabolism. This opens up the possibility for an active role of the embryo in translation of maternal hormonal signals. To understand the role of the embryo, we analyzed steroid dynamics in chicken eggs during early incubation period, comparing the eggs with normal embryonic development to those with experimentally halted development.

Our preliminary data indicate possible involvement of both maternal and embryonic enzymes in early steroid dynamics.

Methods to improve performance and functional traits

7 - Improvement of functional traits and performance in broiler chickens by short-term temperature training in the hatcher

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According to the Agricultural Outlook 2020 poultry meat will be the largest meat sector in the world (OECD-FAO, 2014). To fulfil the growing demand of chicken meat while resources remain limited is only possible with further improvement in production efficiency. On the other side, there is a continuing debate over the risks and ethical questions of commercial poultry farming, which includes animal health and welfare up to food safety and consumer protection in relation to the use of large amounts of antibiotics.
An alternative approach to improve robustness of fast growing broiler chicken lines and with it health, performance and resource efficiency provides temperature training in the hatcher with short-term mild warm loads. During the last days before hatching (perinatal) physiological mechanisms are well developed and regulatory systems evolved from open loop to closed feedback control systems. Temperature training of the developing thermoregulatory system during critical periods has long lasting effects on thermal adaptability and various body functions, because of the strong relationship between the central control of body temperature and body functions, like immune und stress response, reproduction, metabolism, feed intake and body weight regulation. Studies in broiler chickens (Ross 308) under experimental and commercial incubation conditions have shown that short-term temperature training in the hatcher (maximum + 1°C, 2 hrs/day) may increase hatching rate and body weight of hatched chickens, changed the secondary sex ratio in favor to male chickens and improved chick quality (determined by Pasgar©score). In subsequent growth trials of 35 days a better feed conversion mostly along with better body weight gain (up to 3%) were observed (for reference Tzschentke and Halle, 2009: Brit. Poult. Sci. 50: 634-640; Elmehdawi et al., 2015: Brit. Poult. Sci. 56: 381-388). In our experiments, these positive effects were preferentially found in male chickens.
Our hypothesis is that short-term temperature training in the hatcher improves robustness of the chickens, which obviously based on reduced basic metabolism by long-lasting epigenetic metabolic programming. In single eggs oxygen consumption was reduced during the short-term temperature training and body temperature measured in the chorioallantoic fluid on surface of the embryo was also reduced during final incubation. The long-lasting metabolic effect was a lower neuropeptide Y expression in the hypothalamus of prenatally temperature trained chickens in comparison to the control birds. This effect was also preferentially found in males. The improved robustness of perinatally temperature trained broiler chickens is related to more available energy for adaptation, immune and stress responses during environmental challenges, which meet important animal health and welfare as well as food safety aspects. First experiments on stress response in chickens at the end of the growing period confirm this hypothesis. At the end of the growing period, perinatally temperature trained broiler chickens have, for instance, lower heterophil to lymphocyte ratio (HLR) in comparison with the normal incubated control group.
In conclusion, in broiler chickens functional traits alongside with production efficiency may be improved using perinatal temperature training as a practicable epigenetic tool.

8 - The effects of BIOMIN on productive performance and some physiological and immunological traits in broiler chicks

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The objective of the present study was to investigate the effects of Biomin addition as a natural growth promoter in drinking water on some haematological and immunological parameters in broiler chicks.

A total of 180 unsexed, one day old, broiler chicks (Hubbard) were weighed, wing banded and randomly distributed to three equal groups. Chicks in the 1st group were fed a commercial basal diet, watered with fresh tap water and considered as control, while those in the 2nd and 3rd groups were fed the same diet and orally received 20 and 30 mg BIOMIN/ chick during the first three days of age. All chicks were housed in pens under normal and hygienic conditions and exposed daily to 24 continuous lighting hours during the first three days of age. All chicks were provided with feed and water ad libitum up to 42 days of age.

The results obtained we as follows:

1. Adding the tested biological additions (BIOMIN) to broiler chicks increased ($p \leq 0.05$) body weight and body weight gain from 3 to 6 weeks of age in treated groups compared to control group.

2. The treatment with BIOMIN decreased feed intake and improved feed conversion ratio all over the experimental period.

3. No signifiant ($p > 0.05$) differences in hemoglobin, hematocrite (%), total protein, albumin, globulin, total lipids, and glucose was found for chicks in all groups.

4. Total cholesterol level in treated group decreased significantly ($P < 0.05$) at 2 and 4 weeks of age as compared with control group. Creatinine level, AST, ALT and T_3 hormone were not significantly affected by BIOMIN treatment.

5. The humoral immune response against Sheep red blood cell (SRBC,s as Geometric means GM) was increased markedly but not significantly, also the immune response for Newcastle Disease (ND) was not significantly affected with BIOMIN.

9 - Identification and differentiation among chicken, duck, quail, rabbit and turkey meat using PCR-RFLP technique

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PCR–RFLP technique was developed for identification and differentiation among chicken, duck, quail, rabbit and turkey meat. DNA from small amount of muscles (0.05 g) was extracted and a region of mitochondrial DNA (cytochrome-b gene) in chicken, duck, quail, rabbit and turkey was amplified by PCR.

Fragment length of the PCR product was 371 bp in chicken, 374 bp in duck and rabbit and 377 bp in both quail and turkey. Six nucleotides different made it difficult to differentiate among these five species. For differentiation, three different restriction enzymes (DdeI, MspI and TaqI) were used to digest the PCR products. Restriction analysis showed difference among chicken, duck, quail, rabbit and turkey meat. Where, DdeI yielded two fragments (291 and 83 bp) only in rabbit meat, MspI yielded three fragments (221, 85 and 65 bp) in chicken meat and two fragments (290 and 87 bp) in both quail and turkey meat. TaqI yielded three fragments (146, 134 and 94 bp) in duck meat and two fragments (226 and 151 bp) in quail meat.

The use of Cytb-PCR-RFLP assay allowed a direct and fast authentication and differentiation among chicken, duck, quail, rabbit and turkey meat.

10 - Effect of diluents on viability of rooster sperm stored for 24 hours

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Artificial insemination is used in almost 95% of turkey reproductive flocks and is becoming more important in chickens, particularly in broiler breeders. For better work organization and efficient male utilization, diluted semen is recommended for AI practice.

Pooled semen collected twice a week by dorso-abdominal massage from males of two commercial chicken lines, ISA Brown and Hubbard Flex, was divided into four equal parts and diluted 1:2 ratio with four diluents: [1]EK; EK + 1 µg/ml organic selenium (selenomethionine; Sigma - Aldrich) and 8 µg/ml vitamin E (TROLOX, Sigma – Aldrich); EK + 10 mg/ml of Royal Jelly (BARTPOL S.C., Poland) and EK + 0.25 g/ml of lyophilized whey of cow colostrum. Diluted semen was stored for 24 hours at +4°C, in 10 ml tubes. In the freshly collected ejaculates the sperm concentration (with haemocytometer), motility (with Sperm Class Analyzer, version 5.1, Microptic, Barcelona, Spain) and morphology (on the basis of nigrosine-eosin histological smears at 1250x magnification, Nikon Eclipse E100 light microscope) were evaluated, whilst in the freshly diluted semen (after 15 min) and samples stored for 24 h the last two traits were determined.

In both lines semen dilution did not affect ($P>0.05$), in relation to net semen, the number of live normal cells (76.81-81.26% in ISA Brown and 67.79-73.28% in Hubbard Flex) however, colostrum whey addition increased ($P<0.05$) the percentage of bulb head sperm (6.0 vs. 9.81 and 19.05 vs. 13.33, for ISA and Hubbard, respectively) and decreased sperm motility (53.7 vs. 99.1% and 66.1 vs. 98.6% for ISA and Hubbard). The 24 h storage of net and semen diluted with colostrum whey caused ($P<0.05$) unfavourable changes in all evaluated traits in both chicken lines, while semen dilution with remaining diluents decreased the percentage of live normal cells (47.89-58.8 in ISA and 45.74-50.26 in Hubbard); increased the number of dead sperm (from 7.07- 11.56% in unstored ISA semen to 20.10-24.14% after 24 h, and 8.25-13.31% vs. 20.38-23.73% in Hubbard, respectively), but did not affect sperm motility (86.3-92.7% for ISA and 85.1-96.6%).

[1]EK = diluent was elaborated by Ewa Koch (Łukaszewicz); for reference, see Łukaszewicz E. (2002) An effective method for freezing White Italian gander semen. *Theriogenology* 58: 19-27.

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11 - Fine mapping of a growth QTL on chromosome 4 in chicken

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Growth traits are important economic traits in chicken. The identification of quantitative trait loci (QTLs) and their underlying genes will contribute to discover genetic factors affecting chicken growth. Our previous genome-wide QTL analysis in reciprocal F2 crosses between the inbred lines New Hampshire (NHI) and White Leghorn (WL77) revealed a major growth QTL in the distal part of chromosome 4. However, the size of the region is large, 26.9 Mb, where hundreds of genes reside that could potentially affect the growth traits (Nassar et al., 2015, Animal Genetics 46: 441-6). Therefore, we performed fine mapping to physically reduce the chromosomal interval and the number of potential candidate genes.

188 males of generations F10, F11 and F12 from the advanced intercross line (AIL) that has been established from the initial mapping population were used in this experiment. Body weight was measured every 5 weeks until 20 weeks of age and body weight gain was calculated as difference between subsequent body weight measurements. All animals were genotyped using nine single nucleotide polymorphism (SNP) markers at a distance of approximately 1 Mb in the target QTL region on chromosome 4. Association analyses were performed using a linear mixed model. The length of the confidence interval of the QTL region was reduced from 26.9 Mb to 3.4 Mb which contains 30 genes. Markers rs14490774, rs314961352 and rs318175270 had the strongest effect on most growth traits. The data of 60K-SNP Chip, 600K-SNP Chip and DNA sequencing of the parental lines were used to call mutations in the reduced region. The variant effect predictor (VEP) tool was used to predict malfunctions of genes. A total of 489 mutations were identified. A missense variant within ADGRA3 (SIFT=0.02) and a frameshift deletion in a novel gene with the ensemble ID ENSGALG00000014401 were identified. In addition, 5 synonymous variants located in the genes PPARGC1A, ADGRA3, PACRGL, SLIT2 and FAM184B were found. In conclusion, fine mapping of the QTL with an AIL drastically reduced the confidence interval, which supports the identification of causative mutations.

These findings contribute to our understanding of the complex pattern of the growth regulation in chicken.

12 - Whole genome QTL mapping for growth in chicken

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Knowledge of favorable alleles of genes contributing to chicken growth can be used to improve breeding efficiency. As a first step, often a linkage or association study is performed to map genomic loci contributing to the trait of interest.

Reciprocal crosses between the inbred lines New Hampshire (NHI) and White Leghorn (WL77) comprising 579 F2 individuals were used to map quantitative trait loci (QTL) for 24 growth performance and body composition traits (Nassar et al., 2012, 2013, 2015. Journal of Animal Genetics). The lines NHI and WL77 had been selected for high body weight at the age of 20 weeks and for low egg weight during laying period, respectively. Afterwards, the lines were inbred. NHI chickens show a two-fold higher body weight at selection age compared to WL77. Linkage analysis provided evidence for highly significant QTL on GGA1, 2, 4, 5, 7, 10, 12, 15, 26 and 27. The highest QTL effects accounting for 4.6 to 40.2 % of the phenotypic F2 variance were found on the distal region of GGA4 between 42.1 and 88.4 Mb ($F \geq 11.20$). The QTL allele of the high weight NHI line had positive additive effects on body weight, muscle mass and carcass traits ($14.0\ g \leq a \leq 141.8\ g$), and had negative additive effect on adipose tissue mass (-5.3 g). Using body weight as a covariate in the analysis of body composition traits provided evidence for genes in the GGA4 QTL region affecting adipose tissue mass independently of body mass. The QTL effect size differed between sexes and depended on the direction of cross. TBC1D1, CCKAR and PPARGC1A are functional candidate genes in the QTL peak region. The results confirmed known QTL and identified new QTL effects on GGA5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 24, 26, 27 and 28.

Our study confirmed the importance of the distal GGA4 region for chicken growth. The strong effect of the GGA4 QTL makes fine mapping and gene discovery feasible. The final identification of genes contributes to our understanding of the complex inheritance pattern of growth regulations in chickens.