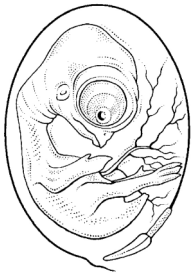
Incubation and Fertility Research Group Meeting



29th and 30th September 2014

Lunteren, the Netherlands

Abstracts (in order of presentations)

Development of the immune response in chicken embryos.

***Silke Rautenschlein**

Clinic for Poultry, University of Veterinary Medicine Hannover, Germany

The immune system is a complex network of cells and molecules, which evolve at different time points during embryonic and post hatch development. Therefore, it takes a few weeks after hatch before chickens have developed a fully mature immune system. This development as well as the immune strategy (humoral or more cellular-oriented) is influenced by the genotype but also by the field exposure to immunomodulatory factors including stress (for example temperature stress, social stress) on the parent and progeny level. Especially viral pathogens as well as colonization pattern of the gut flora, which may be modified by the onset and composition of feeding after hatch, may affect immune development. Almost all immune cells have receptors for one or more of the stress, metabolic, and sex hormones as well as endocrine-signaling molecules. Therefore, these factors have a direct effect on the immune system.

Although we can distinguish the innate and adaptive immune mechanisms in chickens, both systems are closely connected, therefore the immune system may be visualized as an orchestra. Cells involved in the innate immune response, including macrophages, granulocytes, and natural killer cells are oligospecific and recognize mainly evolutionary more conserved pathogen-associated molecular patterns (PAMS) but they do not develop memory. They develop already early in the embryonated egg, and are less susceptible to external influences. Recent studies have suggested that numbers and distribution of innate immune cells in the gut may not be affected by the composition of the gut microflora in young chickens. On the other hand the adaptive immune system is composed of monospecific cells, the B- and T lymphocytes, which express highly variable clonally distributed receptors and develop memory. Although B and T cell development already starts before hatch in the bursa of Fabricius and thymus, respectively, this part of the immune system needs to develop further after hatch. This is influenced by other factors, including colonizing bacteria and possible viruses, as well environmental factors. Immunologically mature cells leave the primary lymphoid organs to home to secondary structures, which includes spleen, mucosa-, pineal- and skin-associated lymphoid tissue. Chickens lack encapsulated lymph nodes, instead they develop diffuse lymphoid tissues at different locations including the gut, respiratory- and reproductive tract. Although these tissues start

developing in predirected sites before hatching, further maturation is antigen-driven and therefore requires contact of the animal to microbes, which mainly takes place in hatched birds, when they are placed in non-germ-free environments. The lymphoid tissue gradually increases with age. Germinal centers, as an indication of antigen-stimulation with aggregation of blast cells, start being detectable around two or eight weeks post hatch in the cecal tonsils and Meckel's diverticulum, respectively, and increase in numbers over time. Interestingly, the gut-associated lymphoid tissue is functionally mature already at four days post hatch, however, the secretory IgA response against enteric antigens develops only gradually, maturing toward the end of fourteen days post hatch.

Maternally derived antibodies protect the developing embryo and neonatal chicken transiently against different pathogens and bacterial toxins. They may persist up to a month after hatch. Immediate immunization after hatch results in a poor active humoral immune response but it significantly increases already at one week of age. The period between two to six weeks can be regarded as an activation phase for the immune system, which is characterized by enhanced expression of proinflammatory cytokines and immunoglobulin, subsequently a more homeostatic phase follows. For some microorganisms, the antibody-mediated (humoral) immunity at mucosal surfaces and/or systemically may be enough to control the invading pathogen, while for others the T-cell-mediated immunity is essential. For some pathogens, the combination of both is necessary for sufficient protection. It may depend on the pathogen and respective field pressure what type of prophylactic strategies should be implemented. Age at time of vaccination may significantly influence the immune response and subsequently protection. While some live vaccines may easily be neutralized by maternal antibodies, other type of vaccines, including immune complex and recombinant vaccines based on a herpesvirus vector may be less affected and therefore can easily be inoculated *in ovo* at 18-days of embryonation or early after hatch. In some countries *in ovo* vaccination is widely used to protect broilers against Marek's disease and sometimes also against infectious bursal disease or other infections. Although not fully developed, embryonated and early post hatch chicks are already capable to mount some specific immunity and innate immune reactions, which help to protect one-day old birds when they are place in the contaminated environment.

Bacterial, viral infections and antibiotic use in the first week

***M.J. Kense, DVM, MAHM**

Animal Health Service - GD, Deventer, the Netherlands

The usage of antimicrobials in the Netherlands in broilers decreased by 50 % in the period 2009-2013. About 35% of this antimicrobial use is prescribed in the first week and about 30-35% is given because of intestinal problems. Reasons for this use are mainly poor chick quality and/or yolk sac infections. The other reason was to prevent problems later on and can be considered as preventive use. This preventive use on farms and also the preventive use of cephalosporin's in hatcheries has stopped in the Netherlands. Bacterial infections or a combination of a bacterial infection with viruses or coccidiosis are important in the level of use of antimicrobials. Bacteria that can be found in hatcheries are mainly *E. faecalis*, *E. coli*, Pseudomonas. In the day-old chick mainly *E. coli* and *E. faecalis* are found. Later on in life different other enterococci like *E. cecorum, E. hirae*, can also play a role often causing their own distinct clinical symptoms. Besides bacteria and coccidiosis, (enteric) viruses can also cause or increase problems. Not much is known about the role of enteric viruses like Rotavirus, Astrovirus, Avian Nephritis virus and REO viruses play in enteric problems and in which they contribute in the severity of other infections. A field trail showed that they all occur in almost every broiler flock with or without causing (subclinical) problems. The age in which they are most present and when they can cause problems differ. It is that clear these enteric viruses play an important role in the use of antibiotics. In order to further reduce the use of antimicrobials, research is needed into the pathogenesis and ways to prevent these viruses to cause problems.

Effects of hatching environment on physiological and immunological development of broiler chickens

***P. de Gouw[1,2], L.J.F. van de Ven[1], B. Kemp[2], H. van den Brand[2]**

[1]Vencomatic BV, PO Box 160, 5520 AD, Eersel, the Netherlands;

[2]Adaptation Physiology Group, Wageningen University, PO Box 338, the Netherlands

Recently new systems have been developed allowing for on-farm hatching of broiler chickens. In on-farm hatching systems, broiler chicken eggs are placed in the broiler house at day 18 of incubation and are hatched in the same house where they will stay for the rest of their lives. Results from practice show higher growth (+1.8%), lower FCR (-3.5%), and lower mortality (-2.2%) in on-farm hatched flocks compared to hatchery hatched flocks. One reason for the different results is the early access to feed and water in on-farm hatching systems. Another reason could be differences in environmental conditions, such as, dust, formaldehyde, and pathogen levels. In previous experiments we found higher concentrations of dust (PM10; 2.2 ± 1.2 mg/m^3) in commercial hatchers compared to on-farm hatching systems (0.47 ± 0.11 mg/m^3). Dust is an important transport pathway for pathogens released during hatch. To combat the spread of (pathogenic) microorganisms formaldehyde is evaporated in the hatcher. Formaldehyde, however, causes lesions in the trachea of day-old chicks. The aim of our experiment was to investigate the effects of these environmental conditions on health and development of broiler chickens in later life. We subjected four groups of broiler chickens to one of four environmental conditions during the hatching phase (day E18-E21), namely control, hatchery dust, formaldehyde, and the combination of hatchery dust and formaldehyde. All broiler chickens were allowed direct access to feed and water. Immunological and physiological development was measured directly after hatch, at transfer to floor pens (day E21), and at day 42 after transfer. At day 42, also, an extensive post-mortem examination was performed to assess slaughter quality. We hypothesize that the environmental conditions will influence the immunological and physiological development and slaughter quality of the broiler chickens. Results will be available at the conference.

Avian sperm cells: morphological and physiological aspects

***M. Marzoni[1], A. R. Taddei[2], A. Castillo[3], F. Batocco[4], A.M. Fausto[4]**

[1]Department of Veterinary Sciences, University of Pisa, Viale delle Piagge 2, 56124 Pisa, Italy;[2] Centre for High Instruments – Electron Microscopy Facility, University of Tuscia, Largo dell'Università, 01100 Viterbo, Italy; [3] External collaborator; [4] Department for Innovation in Biological, Agro-food, and Forest systems, University of Tuscia, Largo dell'Università, 01100 Viterbo, Italy. Email: Email: margherita.marzoni@unipi.it

The spermatozoon has the fundamental task of the fertilization of the ovum, which is the determinant event for the conservation of any species. Knowledge of the ultrastructure of spermatozoa is crucial for the evaluation of basic reproductive biology of species as well as for phylogenetic study and evolutionary relationships. Sperm morphology is a strong indicator of semen quality, as well as sperm concentration and motility, and therefore it is considered a predictor of the fertilizing capacity of sperm. Thus knowledge of it is essential in assisted reproduction procedures, applied for example to solve problems of male subfertility, or for genetic selection programs in animal breeding, or as reproductive strategy in endangered species conservation programs. This presentation aims at reviewing morphology and fine structure of avian spermatozoon, touching on aspects of sperm physiology, and illustrates some main ultrastructural differences observed in spermatozoa from different bird species. The description of sperm morphology and fine structure of two species never examined before, the common pheasant (*Phasianus colchicus*) from the order Galliformes and the goshawk (*Accipiter gentilis*) from the order Falconiformes, is given and comparison with other species considered. Finally, TEM and SEM micrographs of some sperm morphological anomalies observed in fresh semen and some ultrastructural damage caused by cryopreservation are shown.

Apical blebs on sperm-storage tubule epithelial cell microvilli: their release and interaction with resident sperm in the turkey hen oviduct

***Murray R. Bakst[1] and Gary Bauchan[2]**

[1]*Animal Biosciences & Biotechnology Laboratory,* [2]*Electron & Confocal Microscopy Unit, Beltsville Area, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705*

Located at the anterior end of the turkey hen's vagina are numerous discrete tubular invaginations of the surface epithelium, collectively referred to as the sperm-storage tubules (SSTs). Following mating or artificial insemination, sperm ascend the vagina, enter the SSTs, and over ensuing days and weeks, gradually exit the SSTs and are transported to the anterior end of the oviduct to fertilize a daily succession of ova. Little is known regarding the cellular and molecular mechanisms responsible for sperm subsistence in the lumen of the SST. In this study, the origin of microvillus blebs (MvB) on the apical tips of SST epithelial cells and their possible roles in sperm survival are examined. Regardless if sperm are present or not, transmission electron microscopy revealed two types of microvilli differentiated by the presence or absence of pleomorphic, unilaminar MvBs at their apical tips. While some MvBs appeared to be discharging their contents into the SST lumen others appeared to have pinched off the microvillus stem. When SSTs contained clusters of densely packed sperm, the sperm heads of those sperm adjacent to the SST epithelial cell surface were surround by the microvilli. Associated with the plasmalemma of sperm throughout the SST lumina were membrane fragments and small vesicles (30 to 130 nm in diameter) some of which appeared to have fused with sperm. It is concluded that the MvBs are a form of shedding vesicle released from the SST epithelial cell microvilli by apocrine secretion. Based on the observations described herein and those of other authors, it is suggested that the MvBs contribute to sustained sperm storage in the SSTs by (1) serving as a source of metabolic substrates utilized by resident sperm, (2) serving as fusogenic vehicles providing exogenous macromolecules that reversibly suppresses sperm functions associated with fertilization (decapacitation?) and stabilizes the sperm plasmalemma, and (3) serving as transport vesicles actively transporting fluid from the SST epithelial cells to the SST lumen.

Effects of heating of hatching eggs during 4 or 11 days of storage on hatchability, hatching time, and live performance of chicks from young broiler breeder flocks.

R. Shiranjang, S.Özlü, M. Güçbilmez, *O. Elibol, and J. Brake.

Department of Animal Science, Faculty of Agriculture, University of Ankara, Ankara, Turkey, and Prestage Department of Poultry Science, NC State University, Raleigh, NC 27695-7608 USA.

The effects of length of storage period, and heating of eggs during storage, on the hatchability, hatching time, and live performance of chicks from young broiler breeder flocks were examined in this study. Eggs laid in mechanical nests were collected from 28 and 29-wk old Ross 344 X Ross 308 broiler breeders on paper flats, held overnight (1 d) in a hatchery egg storage room at 17C and 75% RH in both Experiments 1 and 2. Eggs were then transferred to plastic setter trays and either remained in the storage room (Control) or were subjected to a heat regimen of 26C for 2 h, 37.8C for 3 h, and 26C for 2 h in a partially filled Petersime setter before being returned to the egg storage room. Eggs were stored for 3 d (Heat 1-4 d) or 10 d more (Heat 1-11 d). Control eggs stored for 4 d or 11 d were co-incubated in each experiment. All eggs were set in a single incubator and hatcher in each experiment. Ten replicate trays of 150 eggs each (1500 eggs total) were set per heating treatment for each storage period in both experiments. In Experiment 2, hatching time was determined by marking all chicks that had hatched (healed navels and dry head and neck) at 468-480 h (Early), 481-492 h (Middle), and 493-510 h (Late) of incubation in 5 trays per heat treatment in each storage period. At 510 h of incubation, all chicks were removed from the trays, feather sexed, counted, permanently identified with neck tags, weighed, placed in floor pens on new wood litter shavings, and subjected to the same feed and management program. For each heat treatment group per storage period, chicks were assigned to 12 replicate pens of 16 male chicks each for a total of 768 chicks. BW and feed consumption were determined at 7, 14, and 21 d of age. Hatchability of fertile eggs decreased with length of egg storage in both experiments due to increased early and late dead embryos. Although no benefit of heating eggs during 4 d of storage (Heat 1-4 d) was observed, heating eggs (Heat 1-11 d) during 11-d of storage reduced early deads and increased hatchability of fertile eggs as compared to control in both experiments. Hatching time was extended in eggs stored

11 d compared to 4 d. However, eggs heated during storage hatched earlier than their Control, which was more evident with longer storage period. Broiler BW and feed intake were significantly decreased at 7, 14, and 21 d of age by longer storage but were not affected by storage heating. Heating eggs during 11 d of storage decreased early deads, increased fertile hatchability of eggs, and reduced late hatching of eggs from young broiler breeder flocks without effect on broiler live performance.

Keywords: egg storage incubation, egg storage time, hatchability, hatch time, body weight, feed intake

Eggshell thickness and hatchability

***Umut Sami Yamak[1], Musa Sarica[1], Mehmet Akif Boz[2]**

[1]Ondokuz Mayis University, Agricultural Faculty, Department of Animal Science, Samsun, Turkey; [2]Bozok University, Faculty of Nature and Agricultural Sciences, Department of Animal Science Yozgat, Turkey

The eggshell plays an important role in incubation, one of the most important factors to affect profitability in poultry production. There are different methods of measuring eggshell thickness. Most common method is measuring with or without membranes using a thickness measurer. However, this method does not sufficiently reflect the effect of shell thickness on hatchability. Hence, researchers have assessed eggshell thickness according to egg specific gravity, which is closely related to shell thickness or have calculated eggshell thickness with a logarithm that used egg weight. However, all these methods assess eggshell thickness indirectly. This paper includes the results of two different experiments. In experiment 1, 253 eggs obtained from broiler breeder flock and in experiment 2, 462 eggs obtained from partridge flock. In both experiments, eggs were collected on the same day, individually numbered and shell thicknesses were measured with an Egg Shell Thickness Gauge (ORKA Tech. Ltd., Israel) that uses precision ultrasound to gauge thickness without breaking the egg and is accurate to within 0.01 mm. Eggs were classified into three shell thickness groups (thin, medium and thick). Eggs were placed in incubator and transferred to individual pedigree hatch baskets at transfer days of incubation. In experiment 1, hatching rates of thin-, medium- and thick-shelled broiler eggs were 91.6%, 84.6% and 91.3%, respectively ($P>0.05$). In experiment 2, hatching rates of thin-, medium- and thick-shelled partridge eggs were 92.9%, 92.3% and 90.8%, respectively ($P>0.05$). No significant differences were observed between groups for hatching rates. The results of two experiments showed that eggshell thickness did not affect hatchability of eggs.

Keywords: Eggshell thickness, incubation, hatchability, broiler, partridge.

Don't underestimate impact of incubation temperature on broiler performance

***Mark Foote**

Hatchery specialist, Cobb Europe. Email: Mark.Foote@cobb-europe.com

This study investigated the effect of different setter temperatures and their impact on broiler performance for two breeds, Cobb500 and another breed (X). The trials were conducted in conjunction with Lagerwey Hatchery, the Netherlands, and HatchTech. Four 4800 egg capacity setters and two commercial setters (57,600 eggs) were used for different incubation temperatures and hatched chicks were followed at Cobb broiler trial facilities in the Netherlands. Eggs from both breeds were used in three separate trials with egg shell temperatures maintained at 100.0°F (37.7°C) for the first seven days of incubation in all trial groups and until transfer to the two commercial setters. For both breeds in two of the small setters temperatures were reduced from 100.0°F at day 7 to 98.5°F (36.9°C) by day 10 until transfer. In the two other small setters temperatures were increased from 100.0°F at day 7 to 101.5°F (38.6°C) (trial 1) and 102.0°F (38.9°C) (trials 2 and 3) by day 10 until transfer. The 2 x 57,600 egg capacity incubators were maintained 100.0°f from setting to transfer. Eggs were weighed before setting and checked for hairline cracks. Probes were attached to designated eggs in positions in the trolley for each setter to record eggshell temperatures every nine minutes during the first 18 days of incubation. Chick length was measured from the tip of the beak to the end of the middle toe with chick stretched out. Chicks were weighed and this value divided by egg weight to express chick yield as a %, and for samples from each trial group heart, intestine and livers were dissected post-mortem, weighed and expressed as a percentage of chick weight.

Hatchability and chicks - Lower incubation temperatures decreased hatchability for both breeds with up to 8% lower (breed X) and over a 1.0% increase cull rates. Hatching times were decreased for both breeds at the higher incubation temperatures although more so for the Cobb 550. For both breeds chick length was greater for early-hatched chicks.

Body composition - The higher the incubation temperature the smaller the chick heart weight. Residual yolk weight was highest for the 98.5 °F group, and lowest for the 100.0 °F. Intestine weight was greatest for 102.0 °F and 100.0 °F groups, whereas liver weight was largest for 100.0 °F groups. For both breeds lower chick yields were associated with lower 7 day mortality but higher 7 day weights.

Broiler results - The 100.0°F groups gave the best weekly bodyweights; the 98.5 °F groups had the lowest bodyweights in the first 21 days, whereas in general the 102.0

°F groups had the smallest weight gains after 21 days of age. The 100.0 °F groups had lowest mortality, while the 98.5 °F groups had the highest mortality in the early stages and the 102.0 °F groups highest mortality in the later stages. The 100.0° °F groups had the lowest FCR at 7 day of age and again at 37 days. The early hatching chicks (102.0°F groups) had the highest FCR.

In summary, eggshell temperatures of 100.0°F produced optimum development of organs and broiler body weights, FCR and mortality. Chick yield (%) can be a useful indicator of optimum incubation conditions and create awareness of potential adverse 7 day body weights and mortality levels when out of the optimum range (66% - 68%).

Effect of lighted incubation from set till hatch on hatch moment and chick development

***C. W. van der Pol[1,2], I. A. M. van Roovert-Reijrink[1], C. M. Maatjens[1,2], B. Kemp[2], H. van den Brand[2]**

[1]HatchTech B.V., P.O. Box 256, 3900 AG Veenendaal, the Netherlands. [2]Adaptation Physiology Group, Wageningen University, P.O. Box 338, 6700 AH Wageningen, the Netherlands. Email presenter: cvdpol@hatchtech.nl

In a commercial practice, eggs are incubated in complete darkness. In nature, a hen leaves the nest regularly, exposing the developing embryo to light. A darkness-light rhythm has previously been shown to influence embryonic brain development, circadian hormonal release, and embryonic development, and it may be speculated that it will influence several other aspects of embryonic development. The data presented here is part of a larger experiment on the effect of light during incubation on bone development. The aim of this part of the experiment was to investigate the effect of light schedules throughout incubation at a constant eggshell temperature (EST) on the hatch window and quality and development of broiler hatchlings. 188 Ross 308 eggs of a 40 week old breeder flock were incubated from day 0 of incubation until hatch using 1 of 3 light schedules: continuous light (24L), continuous darkness (24D), or a 12 hours light, 12 hours darkness daily rhythm (12L:12D). Hatching was monitored every 3 hours to assess the hatch window. Hatched chicks were marked and sampled 3 hours after hatch to determine yolk free body mass (YFBM), navel score, and chick length as measures of chick quality, and heart, liver, stomach (proventriculus and ventriculus), and intestine weight as measures of chick development. 24L chicks hatched earlier than 24D and 12L:12D (-8 and –10 hours, respectively; $P < 0.001$) and had a wider hatch window than 24D and 12L:12D (standard deviation +6 hours for both; $P < 0.001$). Chick length, navel score, and YFBM did not differ between treatments (all $P > 0.19$). Liver weight, corrected for YFBM, was higher for 12L:12D than for 24L (-0.05 g) and 24D (-0.03 g; $P < 0.001$). Intestine weight, corrected for YFBM, was higher for 12L:12D than for 24L (-0.18 g) and 24D (-0.15 g; $P < 0.001$). Heart ($P = 0.11$) and stomach ($P = 0.74$) weight did not differ between treatments.

To conclude, continuous light during incubation (24L) from set until hatch resulted in an earlier and wider hatch window than continuous darkness (24D) or a 12 hours of light, 12 hours of darkness rhythm (12L:12D). However, although the 24L chicks hatched earlier, they were not smaller or of lower quality than the 24D and 12L:12D chicks at 3 hours post-hatch, suggesting their embryonic growth rate may have been increased without a negative effect on day old chick quality. Liver and intestine weight was increase in the 12L:12D group suggesting favourable effects of light/darkness rhythm on internal development.

Long-lasting phenotypic modification due to short-term temperature training during the last days until hatching

***Barbara Tzschentke[1], Ingrid Halle[2], Sabrina Tatge[1], Marleen Boerjan[3]**

[1]Humboldt-University of Berlin, Institute of Biology, Philippstrasse. 13, 10115 Berlin, Germany, [2]Friedrich-Löffler Institute, Federal Research Institute for Animal Health, Institute for Animal Nutrition, Bundesallee 50, 38116 Braunschweig, Germany, [3]Pas Reform Hatchery Technology, Director Research and Development, Bovendorpsstraat 11, 7038 CH, P.O. Box 2, 7038 ZG, Zeddam, The Netherlands.
E-mail: barbara.tzschentke@rz.hu-berlin.de

Environmental experiences during critical periods in the development of regulatory systems may have distinct impact for the lifelong adaptability, health and performance in poultry. Such environmentally-induced phenotypic changes may be even passed on the succeeding generations. In our studies we used a short-term temperature training of a mild increase in incubation temperature by 1°C for 1-2 hrs per day during the last days of incubation until hatching. Such temperature training induces long-lasting changes in thermoregulation as well as in related body functions, such as the control of metabolism, feed intake and body weight. Further, an incubation temperature profile that includes short-term temperature variation is highly relevant to the aim of improving poultry robustness and performance. Results of our previous studies on embryonic temperature training in Ross 308 broilers improved hatchability, chick quality and increased the proportion of hatched male chicks. In incubation trials carried out in a commercial hatchery with short-term temperature training during the last 3 days before hatching (+ 1°C for 1-2 hrs per day) also produced positive effects on hatching rate, chick quality, and an increased body weight of hatched chicks. Important determinants of the impact of embryonic temperature training are long-lasting improvements in performance and adaptability. In growing trials until day 35, an increase in body weight and improved feed conversion were achieved, especially in male Ross 308 broilers hatched from research incubators, as well as a commercial hatchery. The better feed conversion in prenatally temperature stimulated chickens is obviously related to the prenatal imprinting of metabolism to a lower level. Short-term warm stimulation decreased oxygen consumption in broiler chickens during the last days of embryonic development with

long-lasting effects on central nervous regulation of metabolism, feed intake and body weight. Neuropeptide Y (NPY) is one of the most potent orexigenic peptides found in the brain. NPY-expressing hypothalamic neurons play a key role in responding to changes in energy homeostasis by increase or decrease in feed intake. Exclusively temperature-stimulated males showed a significantly ($p<0.05$) lower expression of NPY than conventionally incubated chickens, which corresponded with their significantly better feed conversion and body weight gain. Long-lasting changes due to embryonic temperature training were also found in other body functions controlled by the hypothalamus (e.g. behaviour, reproduction). Preliminary experiments in quail have shown that improvement of hatching-, growing- and laying performance may be even passed to the next generation(s). Further investigations are required to identify the epigenetic mechanisms related to these trans-generational effects.

Cyclically cold incubation temperatures durably affect anti-oxidant pathways and the regulation of energy metabolism in broiler chickens

T. Loyau[1#], *A. Collin[1#], Ç. Yenisey[2], S. Crochet[1], P. B. Siegel[3], M. Akşit[4] and S. Yalçin[5]

[1]INRA, UR83 Recherches Avicoles, F-37380 Nouzilly, France, [2]Adnan Menderes University, Medicine Faculty, 09100 Aydın, Turkey, [3]Virginia Polytechnic Institute and State University, Department of Animal and Poultry Sciences, Blacksburg 24061-0306 USA, [4]Adnan Menderes University, Faculty of Agriculture, Department of Animal Science, 09100 Aydın, Turkey,[5]Ege University, Faculty of Agriculture, Department of Animal Science, 35100 Izmir, Turkey: #Equal contributors

Cyclically cold incubation temperatures improve the resistance of broiler chickens to ascites, yet the underlying mechanisms are not known. 900 eggs obtained from 48 week old Ross broiler breeders were assigned to the following incubation treatments: Control I eggs were incubated at 37.6°C, while Cold I eggs experienced reduced incubation temperature of 36.6°C for 6 h/d from d 10 to 18 of incubation. Chickens were then reared at standard temperatures or under cold exposure, that was either or not associated with a postnatal cold acclimation at d 5 post-hatch. Hepatic catalase activity and malondialdehyde content were measured at hatch. Serum thyroid hormone and triglyceride concentrations, and muscle expression of some genes controlling energy metabolism and oxidative stress were also measured at hatch and at 5 and 25 d posthatch. Cold incubation altered anti-oxidant pathways with higher catalase activity, but lower expression of avian uncoupling protein (UCP3) at hatch. Yet, long-term enhancement in the expression of avian UCP3 was observed, probably caused by an increase in the expression of the transcription factor PGC-1α. An increase in serum T3 concentration was observed only in chickens exposed to both cold incubation and later acclimation at 5 d with cold rearing. Our results suggest that cyclically cold incubation can result in long-term changes in anti-oxidant pathways and energy metabolism, which could improve the health of chickens reared under cold conditions.

Epigenetics and phenotypic variability: insights from birds and long-term effects of embryonic environment in avian species

***Anne Collin[1], Vincent Coustham[1], Laure Frésard[2], Thomas Loyau[1], Mireille Morisson[2], Jean-Michel Brun[2], Tatiana Zerjal[3], Francis Minvielle[3], Aline Bertin[4], Frédérique Pitel[2]**

[1]INRA, UR83 Recherches Avicoles, F-37380 Nouzilly, France, [2]INRA, UMR1388 GenPhySE, 24 Chemin de Borde Rouge, 31326 Castanet-Tolosan, France, [3]INRA, UMR1313 Génétique animale et biologie intégrative, F-78350 Jouy-en-Josas, France, [4]INRA, UMR85 Physiologie de la Reproduction et des Comportements, F-37380 Nouzilly, France.

The chicken genome was one of the first genome of agronomic interest to be sequenced and annotated (although incompletely), and is characterized by a relatively small size, the existence of micro- and macro-chromosomes with contrasting epigenetic properties, and a different sex determinism as compared to mammals. Although epigenetic mechanisms such as DNA methylation, histone modifications and chromatin regulators appear to be conserved in chicken, some mechanisms may be differing compared to mammal species. For instance, the absence of imprinted genes in birds was suggested by several studies.

The avian embryo is a unique model for developmental studies given its potential for *in ovo* manipulation. The embryo is sensitive to environmental influences that can be of maternal (yolk hormones and egg nutrients…) or external origin (incubation conditions including temperature, relative humidity, light colour, gas concentrations...) that have long been known to affect the liveability, development and metabolism of embryos and later chicks. Breeder nutrition and environmental temperature may alter the offspring feeding behaviour, growth or body composition. However, information is scarce about the molecular mechanisms involved in the interaction between environment and the developing embryo. While global and local epigenetic changes have been shown to be involved in the setting of thermoregulation in the chick, the role of such mechanisms in embryo metabolic plasticity and phenotypic variability, and their long term or transgenerational effects remain to be explored.

Modelling and quantification of the thermoregulatory responses of the Developing avian embryo

***Ali Youssef**

KU Leuven, Department of Biosystems, M3-BIORES, Kasteelpark Arenberg 30 bus 2456, Belgium - 3001 Heverlee. Email: Ali.Youssef@biw.kuleuven.be

Homeothermic animals, including birds, try to keep their body temperature at a constant level within certain boundaries by using thermoregulatory mechanisms. However, during incubation, the thermoregulatory system of the chicken embryo evolves through different stages from a poikilothermic to a homeothermic system. Hence, the thermal response of the fertile egg to changes in ambient temperature is different from one day to another during the embryonic development. The incubated egg can be considered as a physical (thermal) system, which transfers energy (heat) down a potential gradient (temperature difference). The heat flow between the micro-environment and the eggshell under a thermal driving force (temperature difference) has been studied in the past by using the analogy to the flow of electric charge under an electromotive-force. In this work, the thermal-response of incubated eggs to a step-increase in ambient-air temperature is studied and modelled. It is shown that the incubated egg is reacting as a first-order system between embryonic days ED01 and ED13, while, starting from ED14, the egg is reacting as a second-order system. This extends the existing RC (Resistor-Capacitor) circuit analogue to an RLC (Resistor-Inductor-Capacitor) circuit analogue at the later stage of incubation. The concept of considering the fertile egg and its surrounding environment as an energy-handling device is introduced in this paper. It is suggested that the thermoregulation of the embryo has a thermal induction-like effect starting from ED14 and increasing gradually until hatching.

Sexing of eggs and adjustment of sex ratio. Can it be used in practice?

***Henri Woelders**

Animal Breeding and Genomics Centre, Wageningen UR Livestock Research, P.O. Box 338, 6700 AH Wageningen, The Netherlands

Sexing of eggs and adjustment of sex ratio would have tremendous impact in the poultry sector for both layers and broilers. In the layer sector, sexing of eggs or adjustment of sex ratio would be used to avoid of decrease the killing of day-old chicks. In the broiler sector, sexing of eggs would allow early segregation of male and female offspring into separate channels of production. This presentation will briefly review recent research on both topics. Recent research showed that sex ratio adjustment in chicken is possible: treatment of the hens with feed restriction, or with corticosterone, resulted in fewer male eggs. These treatments were used to create an experimental animal model to investigate the underlying mechanism of sex ratio adjustment in chicken. We should first find ways to switch on the mechanism in an animal-friendly way before sex ratio change can be considered in practice. Sexing of eggs before incubation would be most ideal, as no developed embryo is killed in that way. Recent research showed that there are differences between male and female eggs, but currently no ready method for sexing eggs before incubation is available. Sexing of eggs before incubation would become a real possibility by using a marker gene on the Z sex chromosome. However, in Europe there may be insufficient public support for a methods that make use of genetic modification. Sexing of incubated eggs, e.g. on day 9 or 10, is possible, but may be difficult or expensive to implement in practice. Apart from technical and economic feasibility, societal acceptability and ethical considerations are important, as people try to weigh arguments such as intrinsic value, utilization, length of life, and ecological footprint.

Poster: Effect of natural and artificial incubation on hatchability and embryonic mortality of geese

***Mehmet Akif Boz[1], Musa Sarica[2], Umut Sami Yamak[2]**

[1]Bozok University, Faculty of Nature and Agricultural Sciences, Department of Animal Science Yozgat, Turkey, [2]Ondokuz Mayis University, Agricultural Faculty, Department of Animal Science, Samsun, Turkey

Goose production in Turkey uses traditional methods, where most of flocks are reared in the backyard. Also, as a part of traditional production, eggs are incubated naturally. As there is increasing demand for goose production in the last few years, commercial goose production farms have been established to meet this demand. As a result of commercialization, the use of artificial incubation has been unavoidable. This study was conducted to determine the effect of natural and artificial incubation on hatchability and embryonic mortalities of geese. 372 eggs were naturally incubated by broody geese. 480 eggs were collected from the flocks of broody geese, and artificial incubation was performed during the same period of natural incubation. In artificial incubation each tray, and in natural incubation each broody goose. were accepted as a replicate and one-way analysis of variance was performed to determine any statistically significant differences. The mean fertility rate of the eggs was 71.62%. There was a significant difference between the hatching rates for the incubation regimens ($P<0.05$). The hatchability of naturally incubated eggs was 91.36%, while the hatchability of artificially incubated eggs was 72.36%. All unhatched eggs were broken to determine the stage of embryonic mortality. There was an insignificant difference between the medium and late period embryonic mortalities among incubations. But, early period embryonic mortalities were significantly higher in artificial incubation (7.92% vs 0.85%; $P<0.05$). In conclusion, natural incubation had better hatching results, but, artificial incubation has to be used to increase the number of total eggs and goslings.

Keywords: Goose, natural and artificial incubation, hatchability, embryonic mortality

This study is a part of first author's doctoral thesis and supported by Ondokuz Mayis University Scientific Research Projects Funding.