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Proceedings of the 2023 Combined Workshop of the European Federation of the World's Poultry Science Association (WPSA) Working Groups 6 (Incubation and Fertility) and 12 (Physiology), Wrocław, Poland, September 18–20, 2023

E. Łukaszewicz[1], **A. Nangsuay**[2] and **B. Tzschentke**[3]

[1] Wroclaw University of Environmental and Life Sciences, Wrocław, Poland

[2] MSD Animal Health, Bangkok, Thailand

[3] Institute for Agricultural and Urban Ecological Projects (IASP) at Humboldt-Universität zu Berlin, Berlin, Germany

Correspondence: ewa.lukaszewicz@upwr.edu.pl, ampai.nangsuay@merck.com, barbara.tzschentke@iasp.hu-berlin.de

Preface

The local organisation of the 2023 Combined Workshop of the Working Groups (WG) 6 (Incubation and Fertility) and 12 (Physiology) of the European Federation of the World's Poultry Science Association (WPSA) was in the hands of Ewa Łukaszewicz and Artur Kowalczyk and their team from the Department of Poultry Breeding at the University of Environmental and Life Sciences in Wrocław and took place under the auspices of the Polish WPSA branch. Co-organisers were the chairs of the WPSA WG 6 Ampai Nangsuay and WG 12 Barbara Tzschentke.

The main objectives of the Combined Workshops, which are organized every two years, are to bring together participants from basic and applied research as well as practice and administration, in order to present and discuss current news and developments around the topic of avian and poultry incubation, reproduction and physiology. Poultry products will continue to dominate the market and secure the population's supply of animal proteins. Improvement in fertility and reproduction provide a significant contribution. Animal welfare is increasingly becoming a decisive competitive factor in poultry production. The course for this is already set in the pre-breeding and hatching phase. Therefore, the orientation of incubation technique and management in line with the physiological needs of the embryos in the various incubation phases is further becoming continuously important. Knowledge of the natural breeding of domestic poultry and wild birds can also make a significant contribution.

We welcomed 100 participants from 16 countries from science, practice and administration to this year's Combined Workshop.

The scientific program offered three invited lectures followed by short presentations thematically organized in seven scientific sessions on topics as fertility and reproduction, embryo communication, egg characteristics, embryonic needs in artificial and natural incubation. The workshop program was concluded with a round table discussion on "Incubation and embryonic needs; effects on performance and wellfare".

The three keynote lectures to the following topics were offered by experts in avian and poultry reproduction, physiology, embryo development and incubation techniques:

- **Anna Hrabia**, University of Agriculture in Krakow, Poland – "Selected molecular mechanisms of structural and functional remodeling of the avian reproductive system",
- **Mylene M. Mariette**, Doñana Biological Station EBD-CSIC, Spain & Deakin University, Australia – "Long-term effects of prenatal sounds in birds",
- **Ron Meijerhof**, Poultry Performance Plus, The Netherlands – "Physics of incubation".

In addition, the Combined Workshop provides students and young scientists a platform to present and discuss their research with a broad audience from academia and practice. In a student competition session for the Nick French Award, the candidate with the best and most original scientific project and presentation was selected. The award was established by Aviagen in honour of Nick French's great contribution to research and practice in the field of poultry embryo development and hatchery science. This year, the Nick French Award was presented to Lotte Hebbink from Pas Reform Academy, NL, for her presentation on "Exploring the impact of external egg characteristics on hatchability".

At this year's Combined Workshop there was also the opportunity to celebrate the 20th Anniversary of WPSA Working Group 12 (Physiology), which was founded in 2003 at the first Workshop at Humboldt-Universität zu Berlin. Barbara Tzschentke, one of the fonders of the WG 12, presented the history, activities and achievements of the WG.

The proceedings are a collection of abstracts of the keynote lectures and short presentations in the individual scientific sessions.

Acknowledgements

We would like to express our special thanks to our valuable sponsors and media partners (Figure 1). We would also like to take this opportunity to thank our honorary patrons, in particular the Ministry of Agriculture and Rural Development, Republic of Poland, the Wroclaw University of Environmental and Life Science, represented by its Rector Prof. Jaroslaw Bosy. Of course, our very special thanks go to the professional organizer VERUS Association as well as all organizing committee members and other persons responsible for the Workshop organization made every effort and have been doing their utmost to make this meeting successful, fruitful and pleasant.

Figure 1. Sponsors and media partners of the 2023 Combined Workshop of the European Federation of the World's Poultry Science Association (WPSA) Working Groups 6 (Incubation and Fertility) and 12 (Physiology).

Session 1 and 2: Fertility and Reproduction

Plenary lecture

Selected molecular mechanisms of structural and functional remodeling of the avian reproductive system

A. Hrabia

Department of Animal Physiology and Endocrinology, University of Agriculture in Krakow, Krakow, Poland

Correspondence: anna.hrabia@urk.edu.pl

During the reproductive cycle (development, laying, pause in laying), the avian ovary and oviduct undergo dynamic morphological and functional changes. After the end of the reproductive season a pause in egg laying occurs, which is accompanied by a decrease in concentrations of plasma ovarian steroids (progesterone, estradiol and testosterone), regression of the reproductive system and its subsequent rejuvenation. Furthermore, within the ovary, follicle growth, development, ovulation and regression caver a series of coordinated processes such as recruitment into preovulatory hierarchy or atresia, extensive angiogenesis and rupture during ovulation. Such events require the extend tissue remodeling which includes alterations in the cell biology (proliferation, death, surviving, differentiation), cell-cell and cell-extracellular matrix (ECM) communication as well as changes in the composition and architecture of the ECM. This review focuses on several molecular mechanisms involved in mentioned processes.

The research from the author's laboratory provided data showing among others: (1) plasma sex steroid profiles during different physiological states of hens; (2) changes in dynamic of cell proliferation and apoptosis and in expression of genes related to these processes in reproductive tissues; (3) alterations in expression of steroid receptor and egg-specific protein genes in the oviduct and liver; (4) participation of aquaporin 4 and connexin 43 in ovarian and oviductal processes; and (5) involvement of matrix metalloproteinases (MMPs) in tissue remodeling of the reproductive system.

Understanding of different molecule implication in molecular mechanisms underlying the reproductive system development and functioning and formation of proper egg in hens may be of considerable importance for poultry production.

Acknowledgement

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Key words

Ovary, oviduct; reproductive cycle; remodeling; birds

Short Communications

Mating behaviour of the Zatorska goose in a harem and in a competitive social structure

M. Gumulka

Department of Animal Reproduction, Anatomy and Genomics, University of Agriculture in Kraków, Krakow, Poland

Correspondence: malgorzata.gumulka@urk.edu.pl

In the natural mating system, the reproductive behaviour of birds plays an important role in fertility success. The aim of the research was to: (1) characterize the mating behaviour of the Zatorska goose in the harem and competitive social structure; (2) determine the relationship between parameters of mating activity (MA), fertility (F) and plasma testosterone (T) concentration. The Zatorska goose is a breed kept in accordance with the roles of the biodiversity conservation program, which makes it a unique research model.

Observations were carried out in the Zatorska goose flock. The birds were kept on litter in pens with harem or competitive mating structure (sex ratio 1 ganders: 4 geese). The recordings were made using a digital recorder connected to the cameras. The MA was described by frequency of courtship displays (Cou), copulation attempts (CopA), successful copulation (SCop) and total mating activity (TMA). In addition, the levels of fertility (artificial incubation) and T concentrations (radioimmunoassay; RIA) were assessed.

In a harem mating system the SCop was 0.65 times/6 h as an average and was higher in the first half of breeding season compared with second half. TMA ranged from 0.66 to 2.36 times/6 h and was higher in the morning than in the afternoon. There was a moderate positive correlation (r = 0.37) for SCop and F. The effect of the position of the ganders in the social hierarchy on the frequency of MA was observed. In dominant ganders, SCop frequency (1.5 times/3 h) was higher than in subordinate ones. The TMA for dominant ganders was 3.6 times/3 h. Plasma T concentration was higher in dominant ganders than in the subordinates. In both MA structures, goose-goose interaction seems to play an important role in mating activation.

Conclusions: It can be assumed that the SCop can be used to identify ganders with poor reproductive potential at the onset of the breeding season. Furthermore, the dynamics of antagonistic gander-gander interactions should be taken into account when creating an optimal sex ratio in small flocks with a competitive social structure.

Key words

Ganders; mating behaviour; fertility; testosterone

Application of assisted reproductive techniques in capercaillie (*Tetrao urogallus*) breeding *ex situ in vivo*

E. Łukaszewicz[1], **A. Kowalczyk**[1], **J. Rosenberger**[1] and **Z. Rzońca**[2]

[1] Wrocław University of Environmental and Life Sciences, Wrocław, Poland

[2] Forestry Wisła, Wisła, Poland

Correspondence: ewa.lukaszewicz@upwr.edu.pl

Many methods used in poultry breeding can be successfully used in the reproduction of wild birds, especially those kept in small isolated populations (zoos and closed, captive breeding centers) and threatened with extinction, as well as for creating an *ex situ in vitro* genetic reserve. The method of semen collection from turkeys and roosters published by BURROWS and QUINN in the 1930 s is now the most widely used techniques for both, domesticated (particularly Galliformes) and free living bird's reproduction.

Since 2008, at the Capercaillie Breeding Center in Wisła Forestry, research has been conducting to improve the reproductive indices of capercaillie kept ex situ in vivo. Using the biotechnological methods known in poultry reproduction we developed/evaluated: 1. two methods of semen collection – dorso-abdominal massage and male stimulation by the female (using the capercaillie phantom) – with the exception of sperm concentration (432.4 x106 ml^{-1} using phantom vs. 614.5 x106 ml^{-1} with the massage), there were no significant quantitative and qualitative differences in semen quality; 2. the quality of semen collected over the entire reproductive season in subsequent years – for the purposes of artificial insemination, the most valuable ejaculates are obtained in the initial and final reproductive period, which is related to female laying period; 3. the environment for short-term semen storage (up to 24 hours at +4°C) – dilution of semen with an EK extender enriched with Se and vit. E, did not cause a significant decrease in percentage of live normal sperm, but sperm motility decreased (by 14.3%); 4. after double insemination

of capercaillies with a dose of approx. 9 million of normal sperm in diluted semen, 90% of fertilized eggs were obtained; 5. two cryopreservation techniques – in straws and pellets without the use of an expensive cry-equipment – the proportion of live normal sperm in thawed semen was similar – 22.4 and 22.2%, respectively, sperm motility frozen in straws was higher than in pellets (46.3 and 27.4%, respectively), but there were no differences in sperm movement parameters; 6. after three inseminations with thawed semen (2–4 days before laying the first egg, the next two every 7 days), with a dose of about 9 million of normal sperm the 84.5% of fertilized eggs on average was obtained (from 77.8 to 91.7%).

Conclusion: Applied methods of assisted reproduction allowed to increase the reproductive indices of the capercaillie and thus the number of birds intended for introduction and the economic effects of breeding.

Key words

Capercaillie; aviary breeding; assisted reproduction

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BURROWS, W.H., J.P. QUINN, 1937: The collection of spermatozoa from the domestic fowl and turkey. Poult. Sci. **16**, 19-24.

Reproductive parameters of capercaillie kept *ex situ in vivo*

A. Kowalczyk[1], **E. Łukaszewicz**[1], **J. Rosenberger**[1], **T. Strzala**[1] and **Z. Rzońca**[2]

[1] University of Environmental and Life Sciences, Wrocław, Poland

[2] Forestry Wisła, Wisła, Poland

Correspondence: ewa.lukaszewicz@upwr.edu.pl

The latest research shows that capercaillie reared *ex situ in vivo* after being released into the natural environment behave like free-living individuals. This observation may have a positive impact on reintroduction success of an extremely endangered species (MERTA et al., 2016). The reproductive traits obtained in captive breeding affect the number of individuals released into nature. The aim of the study was to determine the impact of birds' number in one family on selected reproductive traits.

The experiment was carried out in the Capercaillie Breeding Center in Wisła Forestry in season 2018 and 2019. Two groups of families were created: I – consisting of 2 males and 3–4 females (4 families) and II – consisting of 3 or 5 males and 7–10 females (3 families). Depending on birds' number, one family occupied from 3 to 9 boxes with dimensions of 7.0x4.0 m each. Females of one family could move between boxes through holes with a diameter of 19 cm, while males occupied single boxes (without the possibility of movement). In each group, the following parameters were analyzed: number of set nests, egg fertility and chick hatchability. Genetic material was collected from all individuals of the breeding flock and offspring (including dead embryos) in order to determine chick. To identify each specimen, a set of 29 micro-satellite loci was used. Possessed data were analyzed manually by the confrontation of offspring genotypes with all potential parent–offspring pairs in analyzed

In group I, 66.7% of females set the nest, egg fertility and chick hatchability amounted 65.2% and 83.3%; in group II the following values were obtained: 64.0%, 75.1% and 87.7%, respectively. The genetic analysis showed that, similar to the natural environment where females choose the dominant male, in each family, all offspring were fathered by one male.

Conclusion: Creating the families consisting of 3 or more males allows for a higher egg fertilization rate, however, the participation of only one male in the reproduction indicates that achieving the assumed genetic diversity in the ex situ population may be more difficult.

Acknowledgement

The experiment funded by National Science Centre, Poland, Grant 2016/21/B/NZ9/02084.

Key words

Capercaillie; aviary breeding; reproductive traits; paternity analysis

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Video monitoring as a method of improving the breeding success in breeding centres – the example of capercaillie

J. Rosenberger, **A. Kowalczyk** and **E. Łukaszewicz**

Division of Poultry Breeding, Institute of Animal Breeding, Wroclaw University of Environmental and Life Sciences, Wrocław, Poland

Correspondence: joanna.rosenberger@upwr.edu.pl

The main goal of breeding centres for endangered species, is to produce good quality offspring for release into the wild. To do this, a number of measures are taken to increase breeding success, ranging from optimizing maintenance conditions, to using assisted reproduction techniques. Video monitoring is another tool that can be used with breeding centres to improve those results.

The research was conducted in Capercaillie Breeding Centre in the Wisła Forestry, where birds are kept in conditions similar to natural ones. Females, that are kept in groups one to six, have access to at least two males, allowing them to decide which they will pair with. Females also have access to large fenced yards where they set up the nests and incubate eggs. Both males and females, during the breeding season, are under constant camera surveillance what allow to notice disturbing events (e.g., diseases, presence of predators) and monitoring the course of the reproductive period (mating, incubation).

Thanks to nests 24 hours video monitoring, females behaviour and interactions between them during egg laying and incubation were observed. In four cases two females laid eggs into one nest: in one it resulted in attempts to pull out eggs from the nest and fight between the females (one egg was broken), in two cases nests were abandoned and eggs were taken away by keepers to be incubated in incubator; in the last, despite the change of incubating female, the chicks hatched successfully. Manifestation of aggressive behaviour while another female appears near the nest could be often notice. Usually aggression was limited to the aggressive attitude with raised head and raised feathers on the throat, only occasionally pecking or attempts to push out the individual from the nest. Aggressive behaviour and competition for nest sites negatively affected the breeding success and may induce stress. The frequency of these behaviours increased with the number of birds in family.

Conclusions: The use of video monitoring allows to learn more about problems occurring in captive breed wild animals, and if necessary, to take appropriate decisions.

Acknowledgement

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Key words

breeding success; incubation; video monitoring; capercaillie

Effects of dietary chromium supplementation on performance and chick quality of broiler grandparent hens

M. H. Shahir, **O. Barzegar** and **A. Talebian Soofi**

Department of Animal Science, Faculty of Agriculture, University of Zanjan, Zanjan, Iran

Correspondence: shahir_m@znu.ac.ir

Chromium is a trace element that has shown potential in reducing obesity and improving reproduction in animals. Various studies have demonstrated its effectiveness, particularly in stressful conditions. However, there is a lack of research on the impact of dietary chromium supplementation in broiler grandparent hens.

In this study, 360 broiler grandparent hens were used in a completely randomized design with two dietary treatments and 6 replications (30 hens per replicate). The hens at 34 weeks of age were randomly assigned in two experimental groups and were fed a basal ration or the basal ration supplemented with organic chromium (500 micrograms per kilogram organically chelated chromium). The experimental lasted for 11 weeks, during which daily egg production and weekly body weight were recorded. At end of experiment, 864 eggs from each group were collected and sent to a nearby hatchery for evaluation of hatchability, fertility, and quality of the resulting chicks.

Dietary chromium supplementation increased egg production ($P < 0.05$), egg mass ($P < 0.05$), and reduced feed conversion ratio ($P < 0.05$). The hens that received the chromium supplemented diet tended to weigh less ($P < 0.1$) throughout the experiment. The total hatchability and fertility of the eggs were not affected by the chromium supplementation. Additionally, maternal chromium supplementation led to an increase in the length of chicks ($P < 0.01$).

These results can be attributed to the significant role of chromium in glucose metabolism and its ability to reduce insulin resistance in broiler grandparent hens.

Conclusions: Based on these findings, we recommend dietary chromium supplementation for broiler grandparent hens.

Key words

Chromium; grandparent hens; performance; hatchability; chick quality

The story goes on – update on *in ovo* sex determination

A. Förster

Agri Advanced Technologies GmbH, Visbek, Germany

Correspondence: anke.foerster@agri-at.com

In the ever-evolving landscape of agriculture and animal husbandry, advances are being made to ensure both animal welfare and environmental sustainability. A major issue in the poultry sector is the culling of day-old male chicks from laying lines that do not contribute to egg or meat production and are therefore considered economically unviable. The practice of culling these male chicks immediately after hatch, which has been common for many years, has caused considerable ethical debate. As a result, Germany and France outlawed culling of male day-old as first countries in Europe, others will follow and a European directive is discussed.

To react to the ethical dilemma of culling male chicks in layer hatcheries all over the world, *in ovo* sex determination is the most important alternative, while rearing male chicks and breeding dual-purpose chickens are widely considered as niche-market solutions. For almost 20 years several approaches to determine an embryo's gender before hatch are objects of research. The spectrum ranges from gene editing to various analytical methods of the allantoic fluid (hormone or DNA analysis) and optical methods (spectroscopy, hyperspectral imaging, or magnetic resonance imaging, MRI). One currently market-ready method for *in ovo* sex determination is the hyperspectral imaging with CHEGGY, which is running in several commercial hatcheries all over Europe in a large scale.

For biological and technical reasons, the currently applicable methods determine the embryo`s sex in the second third of incubation, a time point where knowledge about a possible pain sensation of the embryo is limited. In Germany, the law therefore intended that, from 2024, only methods that perform *in ovo* sex determination before the 7th day of development would be permitted. A recent study meanwhile confirmed that the ability to perceive aversive sensory experiences as pain does not seem possible up to and including ED12. This finding led to an adjustment of the law in Germany, so that from 2024 in-ovo sexing techniques can be used before 13th day of incubation. However, a uniform European regulation would be very useful in a common market.

Nevertheless, research on earlier methods continues, as well as investigations to improve the existing techniques.

Key words

in-ovo sex determination; animal welfare; sustainability; pain perception

Nick French Award Competition

Imidacloprid affects calcium homeostasis in chicken spermatozoa

S. Napierkowska[1], **P. Froment**[2], **A. Kowalczyk**[3] and **A. Partyka**[1]

[1] Department of Reproduction and Clinic of Farm Animal, Wroclaw University of Environmental and Life Science, Wrocław, Poland

[2] INRAE, UMR PRC, Nouzilly, France

[3] Institute of Animal Breeding, Wroclaw University of Environmental and Life Science, Wrocław, Poland

Correspondence: skarlet.napierkowska@upwr.edu.pl

The use of pesticides is widespread worldwide, resulting in high concentrations in the environment. Pesticides have been shown to alter Ca^{2+} homeostasis in the cells, mainly by increasing its intracellular concentration above physiological levels. In our experiment, we aimed to test the effect of different concentrations of imidacloprid (IMI) on the calcium homeostasis in chicken spermatozoa.

The experiment was carried out with 10 Greenlegged Partridge roosters. Semen was collected two times per week by the dorso-abdominal massage method and then pooled. Semen was incubated with different concentrations of IMI (0 mM, 0.5 mM, 5 mM, 10 mM, 50 mM) and analysed after 1 and 3hrs. Early apoptosis was determined using Yo-Pro1. Detection of active caspases in apoptotic cells was done using a kit Caspase 3/7 Green Ready, intracellular calcium levels were detected using Fluo-3 AM and propidium iodide (PI). Whereas lipid peroxidation was determined using C_{11}-BODIPY$^{581/591}$ and PI. All analysis were done by flow cytometry.

We found that 1 h after the addition of 0.5 mM IMI, there was an increase in sperm population with low Ca^{2+} level and a decrease in sperm population with high Ca^{2+} level. After 3hrs, the lowest percentage of low Ca^{2+} sperm was still in the control ($P < 0.05$), and in 50 mM IMI. Samples with 0.5 mM and 5 mM IMI contained the lowest ($P < 0.05$) proportion of early apoptotic cells after 1 h of incubation. Only 50 mM of IMI increased the proportion of sperm with active caspases after 1 and 3 hrs of incubation. Lipid peroxidation was significantly highest ($P < 0.05$) in the control after 1 h and 3hrs.

Conclusions: The presence of cells with high Ca^{2+} ion levels in the control versus the IMI samples with low Ca^{2+} ion levels may suggest that low concentrations of IMI block the influx of these ions into the cell. Recent studies show that this is done through the inhibitory effect of pesticides on calcium channel depolarization. The use of different calcium channel blockers would help better understand this mechanism. The detected higher level of lipid peroxidation was associated with an increase in cytosolic Ca^{2+} in the control sperm. Calcium overload can result in formation of Ca^{2+}-dependent pores that increase membrane permeability, leading to induction of early apoptosis in chicken spermatozoa. However, the activation of caspases by 50 mM IMI indicates that IMI does not arrest apoptosis.

Acknowledgement

This research was funded in whole by National Science Centre, Poland, grant number 2021/43/B/NZ9/01550.

Key words

chicken semen; apoptosis; imidacloprid; calcium

Exploring the Impact of External Egg Characteristics on Hatchability

L. Hebbink

Royal Pas Reform, Pas Reform Academy, Zeddam, The Netherlands

Correspondence: hebbink@pasreform.com

There are many variables influencing the hatching success of a fertilized egg. One of these variables is the quality of the egg, which can be divided into internal and external characteristics. High-quality eggs contribute to the overall success and profitability of the hatchery, while impaired egg quality will lead to loss in hatchability. This study focused on the egg characteristics shape index, egg weight, shell colour and shell mottling, which could be measured non-invasively to be able to investigate the relation with hatchability.

Six batches of Ross308 egg with 450 eggs per batch were used. The flock age ranged from 30 to 40 weeks. Before incubation the eggs were all weighed individually (W0), and the shape index was calculated by measuring the height and width. Eggs were categorized into round, normal or sharp eggs. The colour of the shell was assessed visually by using a scale from 1 (brown) to 5 (white). The degree of mottling was visually determined in a dark room with a flashlight, using a scale from 1 (heavy mottled) to 4 (almost no mottling). On embryonic day 18 the eggs were all individually weighed to determine the percentage 18 day weight loss (%WL18). After hatch a break-out was performed to determine true fertility and to determine the developmental phase of the unhatched embryos.

The model used for Hatch of Fertile (HoF) was: HoF = μ + mottling + colour + shape + %WL18 + W0 + batch + e. Where μ = overall mean and e = residual error.

A significant effect ($p < 0.05$) on HoF was found in mottling ($p = 0.0116$) and %WL18 ($p = 0.0153$). HoF was significantly reduced by 5% in heavy mottled eggs of category 1 compared to 2 ($p = 0.0104$). %WL18 had a weak negative correlation with HoF ($r = -0.05$; $p = 0.0059$). All other variables didn't significantly influence hatch of fertile. A second model was used to study weight loss: %WL18 = μ + mottling + colour + shape + W0 + batch + e. All variables except shape had a significant effect on weight loss. %WL18 increased with a higher degree of mottling, lighter shell colour and heavier egg weight. The relation between egg weight loss and mottling might indicate that mottling influences the eggshell conductance.

Conclusions: Hatch of fertile was significantly reduced and weight loss increased in heavily mottled eggs. More research is needed to understand the underlying mechanisms. The shape of the egg (round, normal or sharp) and shell colour had no effect on hatchability in this study.

Key words

Hatchability; egg characteristics; egg weight loss; shell mottling; shell quality

Session 3: Embryo Communication

Plenary lecture

Long-term effects of prenatal sounds in birds

M. M. Mariette

Department of Ecology and Evolution Doñana Biological Station EBD-CSIC Seville, Spain & Deakin University, Geelong, Australia

Correspondence: m.mariette@deakin.edu.au, mylene.mariette@ebd.csic.es

The embryonic stage is a very crucial period of development when many of the organism's functions are set in place. The environmental conditions encountered during that process can shape embryonic development and thereby affect phenotypes on the long-term. While the impact of prenatal nutritional and endocrine environment has long-been studied, the possibility for sound to also shape development has only recently been considered. Yet, sound being so ubiquitous in the environment, it could provide valuable information to finetune embryonic development, or on the contrary – in the case of noise – interfere with their development.

For example, in the zebra finch, a "heat-call" produced by parents incubating in the heat can alter embryonic development, and as a result, adaptively adjust nestling growth to heat. However, the long-term consequences of these changes are only started to be investigated. In this talk, I will present several experiments investigating the long-term effects of embryonic acoustic experience.

In all experiments, eggs were artificially incubated under optimal conditions, and in the last 5 days of development, exposed to playbacks of either heat-calls or control calls. The nestlings were then raised by foster parents, while their growth and physiology was monitored. At adulthood, we tested individual thermal preferences for nest site and microhabitat, as well as their behavioural and physiological thermoregulation capacity.

In addition, to investigate more broadly the sensitivity of embryonic development to sound, we performed a similar experiment where late-stage embryos were instead exposed to playback of either traffic noise or conspecific song.

We found that individuals exposed to heat-calls prenatally choose hotter nest sites in adulthood and alter their microsite use, activity and panting behaviour on hot summer days. They are also more tolerance to heat in adulthood, even though they do not thermoregulate more efficiently. These effects, in addition to the heat-adjusted nestling growth trajectory, may contribute to the observed higher individual reproductive success in adulthood.

On the contrary, noise exposure has detrimental effects on growth, with long-lasting negative consequences on individual physiology and fitness.

Conclusions: Together, these studies demonstrate that prenatal sound has a much larger impact on embryonic development than generally considered, with life-long consequences.

Key words

prenatal acoustic communication; developmental programming; heat adaptation; embryo

Short Communications

Preliminary evidence of an effect of prenatal sound on postnatal thermoregulation in broiler chicks

B.P. Kriengwatana, **O. Günaydin**, **M. Mounir**, **J. Buyse** and **T. Norton**

Animal and Human Health Engineering, KU Leuven, Leuven, Belgium

Correspondence: pralle.kriengwatana@kuleuven.be

Heat stress is a significant problem for poultry health and performance. Approaches to mitigate heat stress in poultry include epigenetic programming by early life exposure to stimulations such as high temperatures and food restriction. In a songbird species (zebra finch) that is adapted to living in hot desert climates, exposure of embryos to adult vocalisations ("heat calls") improves heat tolerance in offspring. Domestic poultry auditory systems also detect and encode sounds during late embryonic development, making it possible that prenatal exposure to specific sounds could be used to efficiently and safely engineer more heat resistant poultry.

Ross 308 broiler chicken embryos were exposed to simulated heat call (*SHC*, n = 30) or control (*CON*, n = 30) sounds in separate incubators for 5 days, starting from embryonic day 17 until hatch. Sounds were played at ~85 dB for 5 minutes every 15 minutes, for 12 hours per day. SHC sounds were shorter, higher frequency sounds with energy concentrated in a few frequency bands that were rapidly repeated whereas *CON* sounds were longer, lower frequency sounds with energy spread out across frequency bands and with longer and variable intervals between sounds. Sound duration, frequency range, and repetition rates were within range of chicken vocalisations. Embryonic heart rate before and during sound playback was measured daily using a non-invasive digital monitor (Buddy Egg Mk2). Incubation temperature and relative humidity of both treatment groups was 37.61°C and 61.7%. At hatch, hatching success, chick quality (Tona score), body weight, and rectal temperature were measured. Body weight and rectal temperature were measured again at postnatal day 7.

Rectal temperature at postnatal day 7, but not at hatch, was statistically significantly elevated in *SHC* chicks compared to *CON* chicks (mean ± SEM SHC = 41.04 ± 0.053°C; CON = 40.86 ± 0.051°C). This effect was likely caused by our treatment altering physiological arousal because heart rate of *SHC* embryos decreased significantly during sound exposure (mean ± SEM before = 241 ± 3.10 bpm; during = 231 ± 3.33 bpm), whereas heart rate of *CON* embryos did not (mean ± SEM before = 239 ± 2.85 bpm; during = 233 ± 3.06 bpm). Hatching success, chick quality, and body weight were not affected by our prenatal sound treatment.

Conclusions: Our data showing increased rectal temperature suggest that rapid, rhythmic, and frequency-modulated high-pitched sounds during incubation could potentially alter thermal tolerance and preference in domestic chickens.

Key words

acoustic developmental programming; rectal temperature; heart rate; heat stress

Acoustic communication between bird embryos – clicking sound and hatch synchronization

B. Tzschentke and **M. Rumpf**

Institute for Agricultural and Urban Ecological Projects (IASP) at Humboldt-Universität zu Berlin, Berlin, Germany

Correspondence: barbara.tzschentke@iasp.hu-berlin.de

Besides vocalization, controlled by the syrinx, clicking sounds, clicks or clicking noise is another kind of acoustic communication between the embryos. They are developed in the last days before hatching, obviously with the onset of respiration, and are audible to the human ear. Embryonic clicking sounds have been demonstrated in turtles and various bird species. Hatching synchronization is widespread in the animal kingdom from insects to birds. The role of clicking sounds in hatching synchronization has only been studied in a few species, e.g. in poultry such as ducks, quails and chickens. This review paper aims to show an overview of the state of the art, including former own research on clicking sound production and hatching synchronization in ducks, and would like to encourage more attention to be paid to this phenomenon in artificial incubation.

Literature search was done using Google Scholar and the electronic data basis of PubMed, Science Direct and Europe PMC. Former own experiments were carried out with Muscovy duck hatching eggs during the last week of incubation, incubated at 37.7 ± 0.3°C and 50–60% rel. air humidity. Clicking sound was recorded non-invasively by microphone at the eggshell. The development of clicking sound with increasing embryonic age until hatch and the relationship of clicking sounds and hatch synchronization under different sound pressure levels (steps of 5 dB) were investigated.

The literature search revealed that, apart from the classic works, there are only a few systematic studies on this topic. The proportion is even smaller when it comes to studies between clicking sound and hatch synchronization and possible mechanisms. Former own systematic investigations in Muscovy ducks have shown, that:

- clicking sounds were produced synchronously with respiration movement
- hatch synchronization by clicking sound depends on ambient noise level; at a noise level of more than 80 dB clicking sound communication was disturbed.

Conclusions: Hatching synchronization by clicking sounds could also be important for commercial poultry incubation. Further systematic studies on the ontogeny and the association of clicking sounds with hatching synchronization in different poultry species and lines, as well as on the role of technical background noise in incubators, are needed.

Key words

incubation temperature; clicks; hatching window

Session 4: Eggs and Egg Characteristics

Variation in capercaillie egg size, pigmentation, and eggshell thickness

J. Rosenberger, **A. Kowalczyk**, **E. Łukaszewicz** and **K. Pytlak**

Division of Poultry Breeding, Institute of Animal Breeding, Wroclaw University of Environmental and Life Sciences, Wrocław, Poland

Correspondence: joanna.rosenberger@upwr.edu.pl

There was found great variation in birds’ eggs, depending factors like life history, genetics or environment. While interspecies variation is well known, interspecies variation is much less described. Aim of conducted studies was to describe egg size, eggshell pigmentation and thickness in relation to female factor, season and laying order.

Unhatched eggs and post-hatched eggshells were collected for three years from two breeding centers in Wisła Forestry District and Leżajsk Forestry District. Date of laying and mother id were noted. From 51 females, 312 eggshells were obtained in total. This includes 171 post-hatched eggshells and 141 eggs infertile or containing embryos up to the 4th day of development. Eggs were measured (maximum length and width) with the use of electronic caliper. The egg shape index, i.e. the ratio of long to short axis, was calculated. Eggshell pigmentation intensity (lightness) was assessed with portable colorimeter NH310. After removing eggshell membranes, eggshell thickness was measured to the nearest 0.001 mm using a micrometer with a 0.2-mm spline diameter.

In the majority of cases, no statistically significant correlation was found between the female id and laying order, eggshell thickness, pigmentation, measures and egg shape. There was no individual variability of the pigmentation of the eggshells in subsequent seasons. We did not found significant differences in eggshell thickness, measures or shape between breeding centers, but eggshells from Leżajsk were lighter. Coefficient of variation showed that females lay similar eggs in clutch in size (CV = 0,041 for length and 0.014 for width), shape (CV = 0.039) and pigmentation (CV = 0,036). The most variable was eggshell thickness (CV = 0.053).

Conclusions: Females lay similar clutches through subsequent breeding seasons. As expected, the greatest variation was found in eggshell thickness, because this egg trait depends very much on external factors, like bird diet, physiological limitations, and environmental conditions.

Acknowledgement

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Key words

egg diversity; eggshell; pigmentation; eggshell thickness

Eggshell ultrastructure in Galliformes and Anseriformes

J. Rosenberger and **K. Pytlak**

Division of Poultry Breeding, Institute of Animal Breeding, Wroclaw University of Environmental and Life Sciences, Wrocław, Poland

Correspondence: joanna.rosenberger@upwr.edu.pl

The eggshell ultrastructure was studied mostly in domesticated species, and the assessment was mainly used for production profitability effect. In wild species, differences were studied mostly in birds with distant phylogenetic affinities and representing extremely different reproductive strategies (altricial and precocial). The purpose of the presented study was to compare the ultrastructure of the mammillary layer in two bird orders (Galliformes and Anseriformes) representing precocials with relatively close phylogenetic affinities, and to relate eggshell ultrastructure to female body weight and egg characteristics (length, width, surface area and volume).

Eight eggs each were collected from nine Galliformes and eight Anseriformes species. From every unfertilized or unincubated egg the 1 cm^2 sample of eggshell was taken from the equator part. Boiling at 5% NaOH for five minutes allowed to expose the surface of the mammillary layer. Photos of the mammillary layer (150x magnification) were done under SEM microscope, EM, Evo LS 15. Statistical analyses were carried out in Python 3.11 Mann-Whitney U test was used to compare the ultrastructural features between orders and validity of the permutation function investigate which traits (female weight, egg parameters) are the best predictors of eggshell ultrastructure.

The number of knobs per mm^2 was higher in Galliformes ($W = 10228$; $p < 0.001$). Percent of knob coverage differs significantly between orders ($W = 6343$; $p < 0.001$) – it was lower in Anseriformes than Galliformes. No significant differences in single knob area were observed between the compared bird orders ($W = 1947.5$; $p = 0.94$). The most important predictors of mammillary layer ultrastructure were bird order, the egg parameters, nor female body weight, were not good predictors of eggshell ultrastructure. Knobs of the mammillary layer function as “storage” of calcium that is necessary for the development of the embryo skeletal system. Based on external appearance Anseriformes are classified as more developed than Galliformes, but this division not be reflected in the ossification of the skeletal system.

Conclusions: Conducted research confirmed that the ultrastructure of the mammillary layer of the eggshell is characteristic of the order, which is related to the ossification pattern.

Key words

Eggshell ultrastructure; Galliformes; Anseriformes; egg characteristics; embryo development

Embryo induced changes of the eggshell – possibilities of egg status determination in wild species

J. Rosenberger, **A. Kowalczyk** and **E. Łukaszewicz**

Division of Poultry Breeding, Institute of Animal Breeding, Wroclaw University of Environmental and Life Sciences, Wrocław, Poland

Correspondence: joanna.rosenberger@upwr.edu.pl

The eggshell plays important role in embryo development, being a protective barrier, allowing gas transpiration and serve as source of calcium for developing embryo. Due to processes that occur during embryo development, a number of changes are observed, mainly in the thickness of the eggshell, and thus its strength and mammillary layer ultrastructure. The aim of conducted research was to identify the most important structural eggshell changes occurred during embryo development on the example of Capercaillie, as so to demonstrate the importance of observing these changes for scientific purposes.

Capercaillie eggshells were collected from two breeding centers – in Wisła and Leżajsk Forestry Districts. The eggshells of 41 unfertilized eggs and 48 posthatched eggshells were selected for comparison of eggshell thickness measured in the equatorial part of the egg. Eggshell from eggs containing embryos that died at different stages of development were prepared for SEM observations (EM, Evo LS 15) by boiling in 5% NaOH for five minutes in order to expose the surface of the mammillary layer. Photos were taken under 150x magnification.

The average thickness in the eggshells where embryonic development was not observed was 0.291 mm and in the posthatched eggshells 0.267 mm. There was a thinning of an average of 8.25% from the initial value. It was observed that from the 8th day of development, small signs of calcium resorption could be seen. Clear signs of calcium resorption on the most of eggshell inner surface were noticeable from the 17th and 18th days of development. From the 23rd day of development, signs of resorption were visible in all taken photographs. In posthatched shells, the knobs of mammillary layer were almost completely dissolved and left no doubt about the status of the egg.

Conclusion: Embryo induced changes in eggshell structure could be used to determine the egg status in case when other methods (i.e. egg content analysis, DNA analysis) is not possible. This method has potential in wild species research where egg content is often removed or decomposed.

Acknowledgement

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Key words

Eggshell ultrastructure; embryo development; Capercaillie

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Session 5: Incubation

Plenary lecture

Physics of incubation

R. Meijerhof

Poultry Performance Plus

Correspondence: ron_meijerhof@hotmail.com

Incubation, the process of successfully transferring the content of an egg into a living day old chick, and it is difficult to even imagine the complexity of the processes that are required inside of the egg to make it happen. However, the success of incubation is, next to the quality of the hatching egg to start with, highly determined by our ability to create the required climatic conditions around the eggs.

To be able to successfully incubate, we have to first of all understand the requirements of the embryo, and then design, set and operate our machines in a way that meets the requirements of the embryo in the most adequate way. To create this optimum environment around the egg, we have to understand the physics that are involved in the process, as physics will determine the environment in the machines.

To understand the requirements of the embryo, in theory we can simply look at the way mother hen does her incubation. With her millions of years of experience, she without any question will know the best what the embryo requires. However, this not necessarily guide us in the way to develop and set the machines, as she uses some different methods to create those conditions compared to our machines with many thousands of eggs. Within our machines, several factors are interacting with each other that are determining the outcome in a way that mother hen doesn't have to worry about. But nevertheless, we can learn some important lessons from her, especially lessons about the importance of conditions for the embryo versus importance of factors that are involved in an adequate functioning of the machines.

If we look at the way mother hen incubates her eggs, it is clear that she only cares about temperature and turning. She doesn't even care about storage conditions, as the first egg from a clutch of 10 eggs will have to wait at least 10 days before she decides that incubation will start, and in that period she doesn't control conditions like storage temperature or humidity.

But once incubation starts, she focusses on keeping the temperature on the required level and turning the eggs on a regular base. In fact, its not only mother hen that regulates the temperature, also the embryo plays an active role by directing more or less blood towards the cooler nest side of the egg (TZSCHENTKE and RUMPF, 2011), in that way fine-tuning the temperature inside of the egg. But mother hen doesn't control factors that are important in our process of artificial incubation like relative humidity (RH) and carbon dioxide (CO_2). This suggests that controlling RH and CO might have more relation with the functioning of the machines than with the requirements of the embryo by itself (MEIJERHOF, 2009; OWEN, 1991).

Requirements of the embryo

Temperature

The most important factor in incubation (besides turning) is temperature (ROMANOFF, 1960). As the embryo only knows the inside of the shell, there is little doubt that the internal temperature of the egg (the embryo temperature or as a reflection of this the egg shell temperature) is the most critical factor in incubation. It is important to realize that the embryo temperature is the result of the balance between heat production and heat loss (LOURENS et al., 2011). This heat loss can be negative (at the start of incubation when the egg needs to take up heat to get to the required temperature) or positive (the embryo produces heat and the egg needs to lose this heat to stay on the required temperature). The heat loss is not only a function of the temperature difference between egg shell and air, but is also highly influenced by the air velocity over the egg and by evaporation of water (MEIJERHOF and VAN BEEK, 1993). RH of the air doesn't have a direct influence on the heat loss of the egg but can influence the evaporation, as in a low relative humidity situation the evaporation of water will be higher.

Temperature in the machine is influenced by the heat production of the embryos, but also by the amount of ventilation, by the temperature and RH of the ingoing and outgoing air (as the heat capacity of air is influenced by these factors), by the amount of evaporation of water and by the effectiveness of the cooling coils. This set of equations can be used to calculate the dynamics of a machine in different conditions (MEIJERHOF, 2009; MEIJERHOF and LOURENS, 2018).

Relative humidity (RH)

The RH in the machine determines the moisture loss of the eggs. In fact, it is not the RH by itself but the difference in water vapor pressure (the water vapor pressure deficit) across the shell (MEIJERHOF and VAN BEEK, 1993). This water vapor pressure is a function of temperature and RH, but as we work with a more or less fixed temperature, we only take RH into account for steering the weight loss of the eggs. The amount of moisture loss is important as the embryo needs a big enough air cell at the end of incubation. The oxygen in the air cell allows the embryo at internal pipping to get the energy that it needs to open the shell and successfully hatch. For successful hatching the total moisture loss (expressed as weight loss of the egg) should be between 12 and 14% of the initial egg weight (AR and RAHN, 1980). As we usually determine the weight loss at transfer time (18 days), we want to have a minimum of 10% but preferably 11–12% weight loss at transfer. This doesn't mean that all the eggs need to lose 11–12% of weight until transfer. There is a huge variation in conductance (approximately 15%) of the shell between individual eggs (VISSCHEDIJK et al., 1985). This means that if the weight loss follows a normal distribution according to the Gauss curve, the standard deviation of weight loss will be 1.5% (15% variation of 10% weight loss). As 99% of the eggs will be in the range of plus and minus 3 × standard deviation, with 10% average weight loss we can expect the eggs with the lowest conductance to lose 5.5% weight, and the eggs with the highest conductance in the batch to lose 14.5% of weight. As a critical lower level of moisture seems to be approximately 6–7% of weight loss (TULLETT, 1990), a total weight loss of 10% during incubation will not be enough to have the last eggs in the safety zone. However, it is good to realize that in that situation not all eggs are in danger, only a few eggs with the lowest conductance will get in problems. But as we want all our eggs to hatch we aim for a higher total weight loss, about 12 to 14%, which means minimum 10% at day 18 of incubation.

Carbon dioxide (CO_2)/oxygen (O_2)

Embryos take up O_2 and produce CO_2 as a result of their metabolic processes. At sea level, it is usually the CO_2, which becomes the first limiting factor for the embryo. The O_2 level is high enough not to create a constraint, but at higher altitudes (above 600–700 m above sea level) it can become limiting (VISSCHEDIJK, 1985).

It is important to understand that there is a relation between temperature and O_2 requirement. As an embryo is poikilotherm, its metabolism increases and decreases with temperature. However, when the metabolism increases due to an increase in temperature, the need for O_2 increases as well. As O_2 must be taken up through the egg shell, an increase in egg shell temperature can quickly lead to a relative shortage of O_2, which makes it more difficult for the embryo to metabolize fat for energy. As an alternative energy source the embryo will then use more protein for energy, which will impair the development of, for instance, the heart tissue, the intestinal track, the leg formation and the immune system, and with that the quality of the chick (MOLENAAR et al, 2010).

The uptake of O_2 and the production of CO_2 are directly related with the metabolism and the heat production of the embryo (ROMIJN and LOKHORST, 1960). An embryo will produce as a maximum approximately 0,15 W of heat, limited by the amount of O_2 available (LOURENS et al, 2007). This determines the production of CO_2, which is at maximum approximately 16–17 ml/egg/hr. Until halfway of the incubation process the CO_2 production of the embryo is much lower (1.25–1.5 ml/egg/hr at 9 days) but then increases exponentially due to the metabolic development of the embryo. After day 14 the heat production is still increasing but not that fast anymore, indicating that the O_2 becomes limiting for the embryo (NANGSUAY et al., 2021).

In modern single stage machine, we usually try to keep the CO_2 level in the machines at a level below 3000–4000 ppm, to not impair the O_2 supply and metabolism of the embryo. Research shows that higher levels up to 6000 ppm do not have a significant negative effect (TULLETT, 1990) but a maximum level of 3000–4000 ppm is at this moment more or less a standard.

Total balance

With these values (CO_2 production and maximum concentration in the machine) together with the level of CO_2 in the incoming air (usually approximately 500 ppm) we can calculate the required ventilation of a machine. For a machine of 100.000 eggs this will be approximately 550–600m3/hr at 18 days of incubation (MEIJERHOF and LOURENS, 2018). When we use this level we can calculate how much heat will be taken out of the machine by ventilation (if we know the condition of the incoming air and the condition of the air in the machine) and how much the RH will be (if we know the RH and temperature of the incoming air). If we want to alter the RH in the machine, we have to either ventilate more (to decrease the RH) or evaporate water in the air (to increase the RH). When the total amount of heat production by the eggs is more than what is taken out by ventilation and by evaporation, the rest will have to be removed by the cooling coils. If the cooling by the cooling coils is not sufficient, either the ventilation or the evaporation has to be increased, which both have a significant impact on the function of the machine in terms of temperature distribution and egg shell temperature.

This means that the level of CO_2 and RH can have a significant effect on the temperature of the eggs in the machine, not because they influence the temperature by itself but because they influence the functioning of the machine in terms of ventilation and spraying. To understand the functioning of the machines, a good understanding of the physics involved in the operation of the machine is beneficial.

Key words

Incubation; physics; embryology; incubator design; incubator operation

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Short Communications

The challenges of artificial incubation used as a conservation tool for threatened wildlife species

E. Arnoux, **A. Jacquet**, **G. Leveque**, **F. Lacroix** and **Y. Hingrat**

Reneco International Wildlife Consultants Research division, Sky Tower, Al Reem Island, Abu Dhabi, UAE

Correspondence: earnoux@reneco.org

Artificial incubation is used in conservation breeding to optimize breeding success of captive endangered birds. However, incubation parameters differ between species and specific parameters such as temperature, humidity, frequency of egg manipulations and egg position inside the incubator (horizontal or vertical) to maximize hatching success and chick viability must be provided.

The Asian Houbara bustards, *Chlamydotis macqueenii*, is an endangered bird bred in captivity for conservation translocation (www.houbarafund.org). Since 1997, numerous experiments have been set to determine the optimal incubation parameters for houbara eggs. Here we summarize main findings of 4 major experiments where several indicators have been assessed: the water loss, the hatchability, the embryonic mortality, the hatching weight, and the chick yield. Successively, we have tested the vertical (n = 298 eggs) *versus* horizontal (n = 298) incubation, the importance of the frequency of egg manipulation with the comparison of all-in all-out incubation method (AIAO n = 513) and three controls of eggs for water loss evaluation during the duration of incubation (n = 512), the temperature inside incubators (37.8 ± 0.1°C n = 400 *versus* 37.6 ± 0.1°C n = 401) and the humidity in hatchers (55% n = 217, 60% n = 213, 65% n = 218, 70% n = 214).

Lower hatchability was observed in the vertical incubation with 69.7% compared to 74.1% hatchability in horizontal incubation. Higher hatchability was recorded for 3 egg manipulations (72.1%) compared to AIAO (60.6%). In addition, the chick quality was better for 3 egg checks (closed umbilic evaluation: 92.3% *versus* 85.2%). The lower temperatures (37.6 ± 0.1°C) during incubation have shown better hatchability results with 75.7% contrasting with

69.6% only for incubation at 37.8 ± 0.1°C. Finally, the four ranges of humidity between 55% to 70% tested in hatchers had no effect on hatchability. 93.2% of viable eggs after 20 days in incubators have hatched and the chick yield obtained was good for all groups (67.7 ± 2.2).

Conclusions: The successive tests for optimization of artificial incubation parameters of houbara eggs have revealed an improvement of hatchability and chick yield. However, the continuous monitoring, evaluation, and finetuning of incubation protocols is crucial to ensure long term optimal breeding success in houbara conservation breeding programs.

Key words

conservation breeding; avian; artificial incubation; incubation parameters

The effect of high EST during the early period of incubation on hatchability and hatch time of long stored broiler hatching eggs

O. Elibol[1], **S. Özlü**[1], **T.A. Şanlı**[1], **T. Erkuş**[2] and **D. Nicholson**[2]

[1] Department of Animal Science, Faculty of Agriculture, Ankara University, Ankara, Türkiye

[2] Aviagen Ltd., Newbridge, Midlothian, United Kingdom

Correspondence: elibol@agri.ankara.edu.tr

During incubation, temperature is the main environmental condition affecting the hatchability and chick quality. The effect of high temperature after the first week of incubation to hatching has been widely investigated. However very little is known during the early period of incubation. This study was conducted to investigate the effect of increased eggshell temperature (EST) during the first 3 embryonic d of incubation (E) on hatchability, percentage of second grade chicks, and hatch time of long stored broiler hatching eggs.

A total of 2700 hatching eggs from Ross 308 at prime flock (39) were collected and stored for 14 d at 15°C in a commercial hatchery. Then eggs were transferred in a climate controlled vehicle to the Ankara University within 2 h. The eggs were then randomly and equally assigned in three identical laboratory incubators each operating at either, a constant temperature of 38.9°C (Group A), gradually decreased from 39.4 to 38.3°C (Group B) or a constant temperature of 37.8°C (Control- Group C) during the first 3 d of incubation. The EST was maintained at 37.8°C from E4 onward in all groups.

No significant difference was found for embryonic mortality and hatchability of fertile eggs among the groups ($P > 0.05$). However, early embryonic mortality was numerically higher in group A compare with the B and C groups. In addition, the percentage of second grade chicks (culls) was tend to be higher in control than the two high EST groups (A and B). Chicks from high EST groups were significantly hatched earlier (6 h) than the control group ($P < 0.05$).

Conclusion: These results suggest that high EST during the first 3d of incubation did not impact hatchability of long stored (14 d) eggs, but chicks hatched earlier (shortened the hatch time) than the control group.

Key words

Incubation; eggshell temperature; hatchability; hatch time

Monitoring temperature and cooling during incubation of geese eggs under field conditions

T. Torma[1] and **M. Rempuszewska**[2]

[1] Intervet hungária kft. – MSD Animal Health Magyarország Budapest, Budapest, Hungary

[2] MSD Polska Sp. z o.o. Poultry Business Unit, Warszawa, Poland

Correspondence: timea.torma@merck.com, malgorzata.rempuszewska@merck.com

The aim of the field measurements to calibrate temperature measuring tools with instant reading devices– Flir thermal camera and Testo laser thermometer – against Tinytag data loggers on goose eggs.

The target temperature of cooling goose eggs during incubation must be determined at the sharp end (apex). Based on the literature, it can vary between 29–30°C (84.2–86^{o}F), therefore both apex and equator were monitored during incubation and cooling with Tinytag, while before and after cooling also Flir and Testo measurements were taken.

Eggs were measured from 12 individual locations, middle and side eggs on the setter tray, on the 15th and 23rd days of incubation.

Based on the results, Flir and Testo can be used to monitor incubation temperature both equator and apex, because their measurement show strong (> 0.85) and significant ($P < 0.01$) correlation with Tinytag data.

However, at the end of the cooling the Tinytag equator showed better point of reference for Flir measurement both equator and apex, both location on the tray, while with Testo only side position, at the equator. The best regression with side egg equator measured with Flir ($R^2 = 0.76$, $P < 0.01$), while including other factors into the equation as, cooling time, cooling temperature and cooling water temperature did not improve the regression, in this field measurement.

Conclusions: The Tinytag logger data showed the temperature difference between the equator and the apex significantly ($P < 0.001$) lower on the eggs on the side of the tray, implying the apex reacts faster on those locations. Based on this, side eggs can be measured with Flir camera to monitor the cooling but preferable at the equator and calculate the desired apex temperature with the following equation:

Apex oF = 0,9609x Equator oF + 2,5437

The position on the trolley does not have impact as we stated in our previous work.

Key words

Goose; incubation; cooling; temperature monitoring

Session 6: Eggs and Natural Brooding

Effects of egg location on the cardboard tray after collection on hatchability of broiler hatching eggs

S. Özlü and **O. Elibol**

Faculty of Agriculture, Department of Animal Science, Ankara University, Ankara, Türkiye

Correspondence: ozlu@ankara.edu.tr

Temperature after oviposition has been found to have a considerable influence on the rate of embryonic development and success of the hatching egg storage. General recommendations for egg cooling after oviposition is that eggs

should be cooled to below physiological zero level within four to eight hours. The rate at which eggs cool is dependent upon a number of factors such as egg size, initial egg temperature, nest and/or storage area conditions and holding time, air movement, collection material, time spent in the cooler. The objectives of this study were to determine the effects of following factors under different egg cooling pattern in a commercial hatchery on hatchability of broiler breeder eggs; 1) flock age (Young-Old flocks), 2) season (Winter-Summer), 3) egg location on the cardboard egg tray after collection (Inner-Outer).

Four experiments were completed to compare the effects of egg location on the cardboard tray after collection. Commercial flocks of Ross 308 broiler breeder were used as the source of broiler hatching eggs. Breeder flock ages were 27 wk (young) and 61 wk (old) in winter and 27 wk (young) and 51 wk (old) in summer season. A total of 2600, 4800, 2600, and 8000 eggs were used in experiment 1, 2, 3, and 4, respectively. All eggs were had been laid within a 15-min period were collected and placed in cardboard egg trays. Eggshell temperature was measured with an infrared thermometer at 30-min intervals during the cooling process. The eggs were divided into two groups, which were inner and outer of the cardboard egg tray and the eggs were marked permanently. All eggs were stored for 5 d at 16°C and 75% RH. At the time of removal of chicks from hatchers (510 h), all unhatched eggs were opened and examined macroscopically to determine, infertile, early, mid, and late embryonic mortality.

The cooling pattern has been affected by location of egg after collection in all experiments, but the effects of egg location on hatchability and embryonic mortalities have been changed by the season and flock age factors. For young flocks, the outer located eggs had a significantly lower hatchability when compared to inner side eggs in winter conditions due to significantly higher late embryonic mortality ($P < 0.05$), whereas in summer season, there was no significant difference between two located eggs ($P > 0.05$). For old flocks, the outer located eggs had a significantly higher hatchability when compared to inner side eggs in summer conditions due to significantly lower early embryonic mortality ($P < 0.05$), whereas in winter season, there was no significant difference between two located eggs ($P > 0.05$).

Conclusions: These results suggest that the egg cooling pattern after lay is an important factor to achieve better hatchability and the cooling pattern might be needed consideration as so flock age and season in case the eggs have been collected to cardboard tray.

Key words

flock age; season; egg location; egg cooling pattern; hatchability

Natural brooding characteristics of the domestic duck

K. Andres[1], **K. Pawlak**[2], **M.W. Lis**[2], **K. Kustra**[2] and **B. Tombarkiewicz**[2]

[1] Department of Animal Reproduction, Anatomy and Genomic, University of Agriculture in Krakow, Kraków, Poland

[2] Department of Zoology and Animal Welfare, University of Agriculture in Krakow, Kraków, Poland

Correspondence: krzysztof.andres@urk.edu.pl

Domestic ducks are one of the main poultry species whose production depends on artificial hatching. Despite improvements in incubation technology, hatchability of duck eggs is lower than that of chicken. The aim of this study was to characterise the natural brooding of the domestic duck using recorders of the incubation environment parameters and broody duck behaviour, serving to better understand the hatching biology of domestic ducks and ultimately to compare with the accepted parameters of artificial hatching.

The study material consisted of pairs of domestic ducks of the landrace variety maintained in pens equipped with nests allowing the placement of loggers recording incubation conditions, i.e. temperature and relative humidity. In addition, during the brooding period, mobile and wireless dummies egg were placed in the nests, which concealed temperature loggers distributed under the surface of the cover at five locations and a centrally located angular acceleration transducer (accelerometer). Data collection was carried out until the end of the brood, after which the data were used to determine natural brood parameters such as temperature during the incubation, the number and duration of nest bouts, the temperature to which the nest was cooled during the nest unattendance, relative humidity and parameters related to egg turning.

The temperature of the nest, as controlled by the loggers, increased with the duration of brooding, while the relative humidity of the nest space decreased. The temperature to which the nestlings cooled the nest took the lowest values during the first week of brooding and the week before hatching. The first half of the incubation period was characterised by a higher number of bouts, while the third week of incubation was characterised by a longer average daily bouts. The activity of egg turning by the brooding duck increased until reaching its highest values in the third week of incubation, after which the frequency of this process decreased.

Conclusions: The natural brooding parameters of the domestic duck obtained using loggers are more heterogeneous than those adopted in artificial incubation technology for this species.

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Key words

domestic waterfowl; incubation; mobile recorders

Session 7: What is essential for embryos?

Development of a non-invasive monitoring system for controlling temperature training in the hatching phase

H.-U. Balzer, **B. Tzschentke** and **N. Petrick**

Institute for Agricultural and Urban Ecological Projects (IASP) at Humboldt-Universität zu Berlin, Berlin, Germany

Correspondence: ullrich.balzer@iasp.hu-berlin.de, barbara.tzschentke@iasp.hu-berlin.de

Temperature training (TT) with short-term mild increase in incubation temperature in the hatcher can lead to acute and long-term improvements in performance and robustness. In order to make this method safer for use in practice, a monitoring and control system is needed that can non-invasively detect changes in physiological parameters of the embryo during TT with high sensitivity and thus enable fine-tuning of the TT.

Temperature training was applied at embryonic days 17 to 20. The incubation temperature (IT) was increased by 1°C for 2 hours per day, starting from the normal IT of 37.3°C. The trials were conducted with Ross 308 hatching eggs (control and TT-group with 30 eggs each) in a hatcher with a capacity of up to 50 eggs (FIEM, MG50H MINILCD, Italy). Highly sensitive temperature sensors of the type EPCOS B57540G with a resolution of 0.01°C have been fixed to the egg shell. It was assumed that the change in heart rate as information about the state of the embryo is hidden in the noise of the measurement signal (change in egg shell temperature, sampling rate 100/sec). The results were used to fine-tune the TT. Based on the hatching results (hatching rate, chick quality, chick weight), after 35 days the growth performance (feed intake, growth rate, body mass), and parameters as mortality, heterophils to lymphocytes ratio (HLR), the success of the fine-tuning of the TT was evaluated.

As physiological parameter periodic changes in heart rate (HR) were recorded and used for TT fine tuning. When exceeding a critical value of the HR (300 b/min) the increase in IT during stimulation was reduced by 0.4 K. The success of this fine tuning could be shown by the recorded production and physiological parameters. The animals that were experienced by TT had a similar good hatching result compared to the control (e.g. hatching rate of 90% in

both groups; chick quality 9.7 ± 0.6 vers. 9.6 ± 0.7) and achieved higher body weights. HLR in the TT-group was improved (0.63 ± 0.30 vers. 0.76 ± 0.46) and in agreement with previous studies. Mortality was zero in both groups.

Conclusion: The developed sensor system for fine tuning of TT could be a method to make TT safer for practice transfer. In a next step a technical solution has to be developed to adapt the method for commercial hatchers.

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Key words

incubation temperature; temperature stimulation; monitoring; temperature sensor; heart rate

Improved broiler hatching results through increased egg-turning frequency during early incubation

B. Machado, **R. Banwell** and **E. Romanini**

Petersime NV., Research and Development, Zulte, Belgium

Correspondence: eduardo.romanini@petersime.com

During incubation, it is normal to turn eggs from left to right once per hour for the physiological function of stimulating embryonic growth and the thermophysical function of facilitating the embryo's heat exchange. In addition, previous research has shown that increased turning frequency during early incubation is crucial for forming sub-embryonic fluid and facilitates the expansion of the vascular area of the chorioallantois membrane (CAM). This study evaluated the effects of increased egg turning frequency during early incubation in experimental and commercial scale trials.

This study was divided into three phases. In Phase 1 (3 experimental scale trials), 2646 hatching eggs/trial from Ross 308 (young, prime and old flock ages) were randomly and equally assigned to three identical laboratory incubators (900 eggs/incubator), as follows: 1) 1 turning/hour from 0–18d (control group); 2) 1 turning/15 min from 0–3d and 1 turning/hour from 4–18d, and 3) 1 turning/15 min from 0–7d and 1 turning/hour from 8–18d. In Phase 2 (commercial scale trials), over 2.8 million eggs from commercial breeds were used to compare the hatching results of a standard turning protocol (1 turning/hour) against an increasing turning frequency during the first week of incubation (1 turning/15 min. from 0–3d and 1 turning/30 min. from 4–7d). In Phase 3, we assessed in 112 eggs the density of blood vessels in the CAM with increased turning frequency to obtain the fraction of vascular area by image processing (4 pictures/egg), according to the methods used by Fernandes et al. (2016).

In Phase 1, increased turning frequency during the first week of incubation consistently reduced late embryonic mortality and improved hatchability in all flock ages. Similarly, in Phase 2, the increase in the turning frequency resulted in average gains of 0.3% in egg weight loss and hatchability by mainly reducing late mortality. In Phase 3, the percentage of area with blood vessels in the CAM increased by approximately 2% on average on embryos at 11 days of incubation with increased turning frequency.

Conclusions: Overall, the results demonstrate the advantages of reducing late embryonic mortality and malposition of embryos by increasing the turning frequency of eggs during the first week of incubation. These results were similar in the commercial scale trials, confirming the increase in hatchability. In addition, there was an increased egg weight loss, partially attributed to greater blood vessel vascularisation.

Key words

Hatchability; late embryonic mortality; malposition; egg weight loss; blood vessel density