ifrg 2021

INCUBATION & FERTILITY RESEARCH GROUP

2021 MEETING ABSTRACTS

Can incubation temperature and early feeding affect later life resilience of broilers?

Jan H. Wijnen[1,2], Carla W. van der Pol[1], Henry van den Brand[2] and Roos Molenaar[2]
[1] Research Department, HatchTech BV, PO Box 256, 3900 AG Veenendaal, the Netherlands
[2] Adaptation Physiology Group, Wageningen University, PO Box 338, 6700 AH Wageningen, the Netherlands

Antimicrobial resistance threats have resulted in a rising interest in non-antibiotic approaches to support broiler health. A promising approach is to enhance broiler resilience.

Resilient broilers are able to cope with stressors during life with minimal loss of function. Broiler resilience might be enhanced by optimising conditions during critical windows in early life, such as incubation conditions and neonatal feeding conditions. Lowering eggshell temperature (EST) during late incubation and early feeding are found to alter chick quality at hatch and post-hatch immune responses.

However, it has not been studied yet whether or not these alterations are related to modulated broiler disease resilience.

An experiment was conducted with a 2×2 factorial arrangement to study effects of EST during late incubation (embryonic day 17-19.5; 37.8°C (control) or 36.7°C (lower)) and feeding strategy (immediate access to feed and water after hatch (early feeding) or delayed access by 48 hours (delayed feeding)) on broiler chicken resilience.

Eggs (n=1,400) of a 54-week-old Ross 308 breeder flock were incubated. After pull, 960 broilers of both sexes were equally divided over 32 floor pens (eight replicates per treatment) and reared for five weeks.

Necrotic enteritis was induced by a single inoculation of Eimeria spp. at day 21 and repeated Clostridium perfringens inoculation (3x/day) during day 21-25. Mortality and body weight change were measured daily as indicators of resilience for 14 days post Eimeria inoculation (PEI).

Additionally, disease morbidity was assessed in intestines (necrotic lesions, coccidiosis, dysbacteriosis), faeces (shedding of oocysts), and feet (footpad dermatitis). No interaction between EST and feeding strategy was found for any variable. Lower EST resulted in more body weight loss ($P=0.02$) and more oocysts in faeces at day eight PEI ($P<0.01$) compared to control EST. Mortality was not affected by EST. Early feeding tended to lower mortality ($P=0.06$; $\Delta=6.6\%$) and lowered incidence of footpad dermatitis in females, but not in males at day 6 PEI ($P=0.04$; $\Delta=13.0\%$) compared to delayed feeding. Feeding strategy had no effect on body weight loss. Morbidity characteristics were not affected by EST or feeding strategy.

In conclusion, it appears that a lower EST of 36.7°C during late incubation impairs broiler resilience to necrotic enteritis at four weeks of age. Early fed broilers tend to have lower mortality after necrotic enteritis induction, which may indicate improved resilience. However, findings were not manifested consistently in all parameters that were measured, and conclusions are drawn with some restraint. ■

jan.wijnen@wur.nl

The effect of different modelling approaches to account for evaporative moisture loss on embryonic mortalities in ostrich eggs

Zanell Brand[1] and Schalk W.P. Cloete[2]
[1]Directorate: Animal Sciences, Western Cape Department of Agriculture, South Africa
[2]Department of Animal Sciences, Stellenbosch University, South Africa

Accounting for variation associated with evaporative moisture loss (ML) could affect genetic parameters for embryo mortality traits in the following two ways:

- Modelling for ML could explain some of the variations between families, resulting in a slight reduction in the genetic variances and heritability estimates.
- In contrast, it could reduce the environmental variance component, resulting in an increase in the genetic variances relative to the phenotypic variance and a higher heritability.

Against this background, the genetic variances associated with incubation traits and embryo mortality (EM) were analysed, using two modelling approaches, namely a quadratic polynomial or a cubic spline, to describe the impact of evaporative ML on these sources of hatching failure.

Overall, EM was affected by hatching year, means ranging from 0.240±0.017 in 2002 to 0.613±0.026 in 2012.

Seasonal changes in early and late EM resulted in means for overall EM increasing from 0.358±0.014 in winter to 0.398±0.014 in spring and to 0.434±0.015 in summer.

Female age markedly affected EM with the lowest losses in eggs produced by two-year-old females and the highest in nine+-year-old females. Eggs with low and high ML sustained higher EM as modelled with a quadratic polynomial.

A cubic spline confirmed the effect of age on EM, but age differences were less evident at lower ML levels.

Direct heritability estimates for the weight and ML traits ranged from 0.23-0.29. The maternal genetic effects were larger for weight traits at 0.34-0.38, but lower for moisture loss traits at 0.14-0.18.

In the case of EM, direct heritability estimates were lower, ranging from 0.09 for early EM to 0.14 for overall EM.

EM was independent of maternal genetic effects, but dam permanent environmental effects ranged from 0.03-0.05 depending on the EM trait.

Heritability estimates for embryonic losses in ostriches were independent of whether the impact of evaporative ML was accounted for or not.

As a matter of fact, heritability estimates derived with models including the effect of ML were consistently lower in absolute terms than models where ML was not considered.

It may thus be unnecessary to consider ML in analyses on determining the heritability of ML. ■

zanellb@elsenburg.com

Incubation & Fertility research group

2021 Meeting Abstracts

Fast embryos-slow embryos: using chickens to understand 'developmental tempo'

Megan G. Davey
Functional Genetics and Development, The Roslin Institute, University of Edinburgh, Easter Bush, Midlothian, EH25 9RG, Scotland, UK

Embryonic development is a process of progressive steps, which lead to the generation of patterned structures. While the sequence of developmental events is highly conserved between species, the speed or 'tempo' at which they occur is a unique feature of a species and highly variable.

Mice embryos, for example, develop at a much faster rate than human embryos; the internal molecular clocks in developing mice embryos have been shown to tick much faster. We hypothesise that in chicken lines which have been subject to rigorous genetic selection for post hatch growth traits, developmental tempo has also been altered and that this in turn may alter duration of incubation time to hatch. Selected chicken lines might be an invaluable resource in which to understand the genetics which control embryonic tempo in all species. Using comparisons between avian species and between chicken breeds, we have worked to establish a 'tempo phenotype'. which could be used routinely to examine this pre-hatch trait. Embryonic tempo is a relatively unexplored field presenting an opportunity to investigate and develop a fundamental area of biology with far reaching implications for many fields, including selective breeding. ■

megan.davey@roslin.ed.ac.uk

Transcriptome analysis of single insemination sperm storage tubules throughout the duration of fertility

Kristen Brady and Julie A. Long
Animal Biosciences and Biotechnology Laboratory, BARC, ARS, USDA, Beltsville, MD 20705, United States

Sperm storage tubules are specialised invaginations of the oviductal epithelium that permit avian species to store spermatozoa for extended periods of time, without compromising sperm fertilisation capacity.

The molecular and physiological mechanisms behind sperm storage tubule differentiation, sperm protection, and regression remain largely unknown and have potential implications in drastically improving successful sperm storage and cryopreservation in commercial poultry species. RNA sequencing was performed on sperm storage tubules isolated from the oviductal epithelium from inseminated hens at day 1, 7, 30, 60, and 90 post-insemination (n=4 per timepoint).

Read mapping and differential expression analysis were performed using CLC Genomics Workbench. A total of 1,910 differentially expressed genes were subjected to enrichment analysis and pathway analysis through DAVID and Ingenuity Pathway Analysis (IPA), respectively.

RT-qPCR analysis was utilised to confirm the results obtained through RNA sequencing. Through characterisation and pathway analysis of differentially expressed genes during early, peak, and late egg production, novel insights into the role of collagen and eosinophil-mediated epithelial remodelling in the formation and regression of sperm storage tubules, of immune pathways associated with the duration of fertility, and of cholesterol and lipid metabolism in sperm protection were identified.

Upstream analysis identified potential regulatory roles for GATA binding protein 3 (GATA3), progesterone receptor (PGR), and interleukin 5 (IL5) in sperm storage tubule function and sperm mobility reduction. Understanding sperm storage tubule function throughout the laying cycle and in terms of sperm preservation, may allow for the development of industry-based protocols for semen storage and cryopreservation that manipulate the sperm preservation capabilities of sperm storage tubules. ■

kristen.brady@usda.gov

In ovo sex determination – the current status and a closer insight

Förster Anke
Agri Advanced Technologies GmbH, 49429 Visbek, Germany

In many European countries, efforts are being made to find welfare-friendly and socially accepted alternatives to the killing of male day-old chicks from layer strains.

For example, in Germany and France laws have been passed to ban the killing of chicks from January 2022. Besides the breeding of dual purpose lines and the rearing of the male 'brothers', in ovo sex determination is an important possibility to overcome the long-standing practice. For more than 15 years, several approaches to determine a chicken embryo's gender have been objects of research.

Commercially applicable methods have to be not only accurate (low error rate), but also meet the requirements of modern hatcheries with a high level of throughput of hatching eggs.

Essentially, two approaches can be distinguished: on the one hand, the liquid-based methods, which differentiate female and male embryos based on 'biomarkers' (hormone derivatives, DNA, proteins) in the allantoic fluid, which is collected through a hole in the eggshell. Optical methods, on the other hand, determine the sex and developmental state of the embryo contactless using light (spectroscopy, hyperspectral analysis).

Based on the sexual dimorphism in plumage colour in brown egg strains, the method of Hyperspectral Imaging detects differences between brown female and yellow-white male chicks non-invasively through the intact eggshell with high accuracy on day 13 of incubation.

This method combines a high throughput rate of around 20,000 eggs per hour with low impact on hatchability and low sexing error rate, so only 8% more hatching eggs are needed (2.6 eggs/female chick compared to 2.4 eggs/female chick without in ovo sexing). For biological and technical reasons, the currently applicable methods can only show gender in the second third of incubation, a time point where knowledge about a possible pain sensation of the embryo is very limited.

For this reason, a method for the electrical stunning of embryos in the egg was developed. With this process it is possible to eliminate pain sensation that may be present at the termination of development with a very high degree of certainty.

Further research is focused on gender sorting before day seven and laboratory results are promising. As of today, no technical viable large scale technology is available which can be used before day seven. ■

anke.foerster@agri-at.com

Relation between air and eggshell temperatures in small-size egg incubators

Mohamed I. El Sabry[1], Mohamed M. Ibrahim[2] and Barbara Tzschentke[3]
[1]Animal Production Department, Faculty of Agriculture, Cairo University, 12613, Giza, Egypt
[2]Agricultural Engineering Department, Faculty of Agriculture, Cairo University, 12613, Giza, Egypt
[3]Institute for Agricultural and Urban Ecological Projects (IASP) at Humboldt-Universität zu Berlin, Philippstr. 13, 10115 Berlin, Germany

Poultry production is an important source of income for small stakeholders, especially, in lower-income countries.

Developing hatchery practices through increasing the efficacy of small-size incubators would improve the living standard of small stakeholders. Temperature is the foremost environmental factor affecting the livability of avian embryos.

In parallel, the heat distribution inside the incubator is critical for embryonic development and producing healthy chicks. The effect of the incubator temperature on egg temperature has been studied, but not well determined or defined in small-size incubators.

Also, the role of air circulation as a factor that affects heat distribution is not well described in small-scale machines.

Electronic tools and software could be efficient in illustrating the problem and can help in finding a solution.

Thus, this study was conducted to determine the relation between heat distribution and the eggshell temperature as well as illustrate the influence of airflow on the temperature inside a small-size incubator.

Two small-size incubators (capacity of 950 eggs each) were used to incubate four batches of hatching eggs. During the first 18 days of the incubation period, the incubator temperature and the eggshell temperature were measured at different points in each incubator cabin.

Then, contour plans for heat distribution and eggshell temperature were plotted using Surfer data-mapping program (Version 9.1.352) (copyright 1993-2009, Golden Software Inc).

The results of heat distribution inside the cabin revealed that the temperature gradually declined from top to bottom levels and varied between different positions at the same level.

Similarly, the eggshell temperature varied between different levels and among the same level.

The regression coefficient of eggshell temperature on incubator temperature indicated that incubation temperature and heat distribution had a significant influence on eggshell temperature.

It could be concluded that:

- Improper heat distribution could be found in a small-size incubator as in the big-size ones.
- Using the new technique to visualise the problem of uneven heat distribution inside the incubator cabin could help in improving the design of egg incubators to provide the optimum growing condition for embryos.

Finally, improving the performance of small-size incubators can also help to improve the quality of life of small stakeholders in developing countries. ■

m.elsabry@daad-alumni.de

In ovo sexing and its rapid expansion

Wouter Bruins
In Ovo, Pesthuislaan 8, 2333 BA, the Netherlands

Every year 6.5 billion layer chicks are culled in the poultry industry. These are male, unable to lay eggs and thus useless in egg production. This complex problem touches on several dimensions, such as ethics, efficiency issues and business. In the past years, several research groups and companies have been developing technologies to gender type the egg, instead of the chick. Thereby hatcheries can choose not to hatch the male eggs, thus reducing culling of male chicks.

Important parameters to consider in a successful gender typing technology include incubation day of testing, capacity of the process and cost per test. As hatcheries are typically built for a relatively long period of around 30 years, the in ovo sexing method will need to be retrofitted and can thus not disrupt the quality of traditional production.

One of the technologies which entered the market in the last year is the Ella system, a high throughput Mass Spectrometry based platform, developed by In Ovo, the Netherlands. This consists of an autosampler, capable of taking small samples from eggs at day nine of incubation. It uses a fast Mass Spec system to detect the concentration of a biomarker called Sabine Amine, which was discovered in collaboration with Leiden University and the Leiden Academic Centre for Drug Research. The machine has an overall capacity of over 11 million tests annually, operating within normal working weeks and requires two people to operate.

The culling of male chicks is an issue for some decades and now that alternatives are production ready, it is expected that these will rapidly expand in the European market. As Germany has banned the culling of male chicks from January 1st 2022 and France by 2023, there is a strong drive towards adoption of in ovo sexing in the egg production market. As all technologies develop further and the market opens up, capacity for in ovo sexing is probably increasing and the amount of culled male chicks will be lowered significantly in the coming years. ■

wouter@inovo.nl

Profiling the emission of volatile organic compounds in the first half of incubation

Matthias Corion, Bart De Ketelaere, Maarten Hertog and Jeroen Lammertyn
Department of Biosystems, Division of Mechatronics, Biostatistics and Sensors, KU Leuven, Belgium

During recent years, research has been performed on the emission of egg gases. These so-called volatile organic compounds (VOCs) demonstrated their potential towards gender identification, fertility discrimination or breed distinction.

Although a wide range of compounds from different chemical classes has been identified, profiling of these gases during incubation has not yet been investigated as such. Therefore, the purpose of this study was to investigate the emission of VOCs throughout incubation.

Gas chromatography-mass spectrometry (GC-MS) was used for this purpose, from day 0 until day 12 of incubation. Identifying these specific gases and how they evolve during incubation carries potential in linking them to biochemical and physiological processes that occur during embryonic development.

For the experiment, 50 eggs were incubated under standard conditions (37.7°C, 45% RH). During each of the even days of incubation, a set of seven eggs was removed from the incubator.

Each egg was then individually incubated at 37.7°C by placing it in a closed glass jar for one hour, followed by 70 minutes extraction using a solid-phase microextraction fibre.

Next, the fibre was desorbed, followed by a one hour GC-MS analysis. Extensive identification resulted in a total of 65 appearing compounds. Here, a principal components analysis (PCA) and a partial least squares regression (PLSR) showed that a clear change in volatile profile was present from day 0 until day 12. Furthermore, a K-means cluster analysis divided the gases into five distinct clusters dominated by specific chemical classes: acids, aromatics, aldehydes, alcohols and alkanes.

Each of the clusters had a particular time-dependent pattern whereby the majority of the compounds decreased over time and the acids increased in their concentration towards day 12. Biochemically, it is known that fatty acids and amino acids are degraded into aldehydes, alcohols and acids.

A proportional increase of acids relative to the proportional decrease of alcohols and aldehydes, after four and six days respectively, suggests that lower oxygen concentrations later in incubation might result in a shift towards acid production.

Finally, the aromatics cluster tends to have its highest proportion at day 0 with a subsequent decrease over time. This suggests the presence of potentially xenobiotic compounds that were absorbed by the egg during or after laying and were subsequently re-emitted or metabolised during incubation. The biological relevance of egg gases has been demonstrated in previous research. This study provides a dynamic pattern of these gases throughout the first 12 days of incubation.

A clear distinction over time was observed together with specific clustering of chemical classes demonstrating their potential in linking and interpreting biochemical and physiological events during embryonic development. ■

matthias.corion@kuleuven.be