ifrg Incubation & Fertility Research Group

THE WORLD'S POULTRY SCIENCE ASSOCIATION

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49th IFRG MEETING

Limak Limra Hotel & Resort, Antalya, Türkiye

ifrg Incubation & Fertility Research Group

THE WORLD'S POULTRY SCIENCE ASSOCIATION

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WELCOME
49th IFRG MEETING

October 3rd-4th, 2024
Limak Limra Hotel & Resort, Antalya, Türkiye

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Limak Limra Hotel & Resort, Antalya, Türkiye

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Dr. Serdar Özlü - Ankara University, Türkiye
Dr. Ron Meijerhof - Poultry Performance Plus, The Netherlands
Dr. Barbara Tzschentke - IASP at Humboldt-Universität zu Berlin, Germany

Preface IFRG

Dear egg and chicken lovers,

We are pleased to welcome you to the 2024 IFRG meeting!

The exciting world of fertilization and incubation has its own research group, the IFRG (Incubation and Fertility Research Group), which meets once a year. This is working group six (WG6) under the umbrella of the European Federation, WPSA (World's Poultry Science Association), which provides a platform where people who are active in research in industry and/or the academic world can meet and exchange ideas and experiences, share results, discuss common interests, and enjoy the company of people with similar passions.

An important objective of the IFRG is not only to share information between people who have work experience in this field but also to inspire young scientists to share their works within the group and challenge the existing knowledge.

This year, the 49th edition of the IFRG is held in Antalya, Türkiye. Approximately 80 delegates from all over the world will join to discuss various topics, ranging from fertility, egg storage, and treatment to incubation conditions of several avian species eggs, data analysis, egg hygiene, and other management-related topics.

The program contains a mixture of sciences and field experiences, with a number of well-known experts addressing specific topics in keynote presentations, **Rooster Fertility: Diagnosis and Conservation, SPIDES - Turning Science into Practice and Impact of Incubation Temperature on Meat Quality in Broilers.** On the first day, we will have the Nick French Prize session for students under the age of 32 to win the best research and presentation award.

This year, we started the IFRG NextGen Funding to give young scientists opportunities and nurture their future. We would like to express our appreciation to Poultry Performance Plus, Hendrix Genetics, and Ampai Nangsuay for their sponsorship. Our appreciation also goes to Aviagen for sponsoring the Nick French Prize, which was established in 2019. Moreover, this meeting cannot exist without the support of Aviagen, HatchTech, Petersime, Royal Pas Reform, MSD Animal Health, Agri Advanced Technologies GmbH, HIPRA and VISCON of which we are very grateful.

In addition to learning and sharing knowledge about incubation and fertility during the meetings, we hope that you will take time to enjoy Antalya, where nature's colors, blue and green, unite!

We hope you all enjoy these coming days!

Organizing Committee

Dr. Ampai Nangsuay
Dr. Roos Molenaar
Dr. Serdar Özlü
Dr. Ron Meijerhof

Meet the Keynote Speakers

Anais Carvalho, Ph.D

Topic

Rooster Fertility: Diagnosis and Conservation

Dinah Nicholson, Ph.D

Topic

SPIDES - Turning Science into Practice

Servet Yalçın, Ph.D

Topic

Impact of Incubation Temperature on Meat Quality in Broilers

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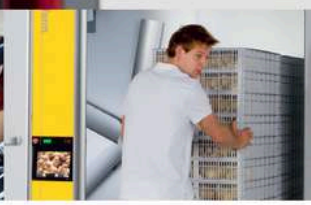

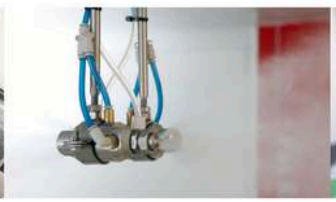

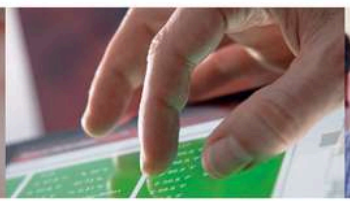

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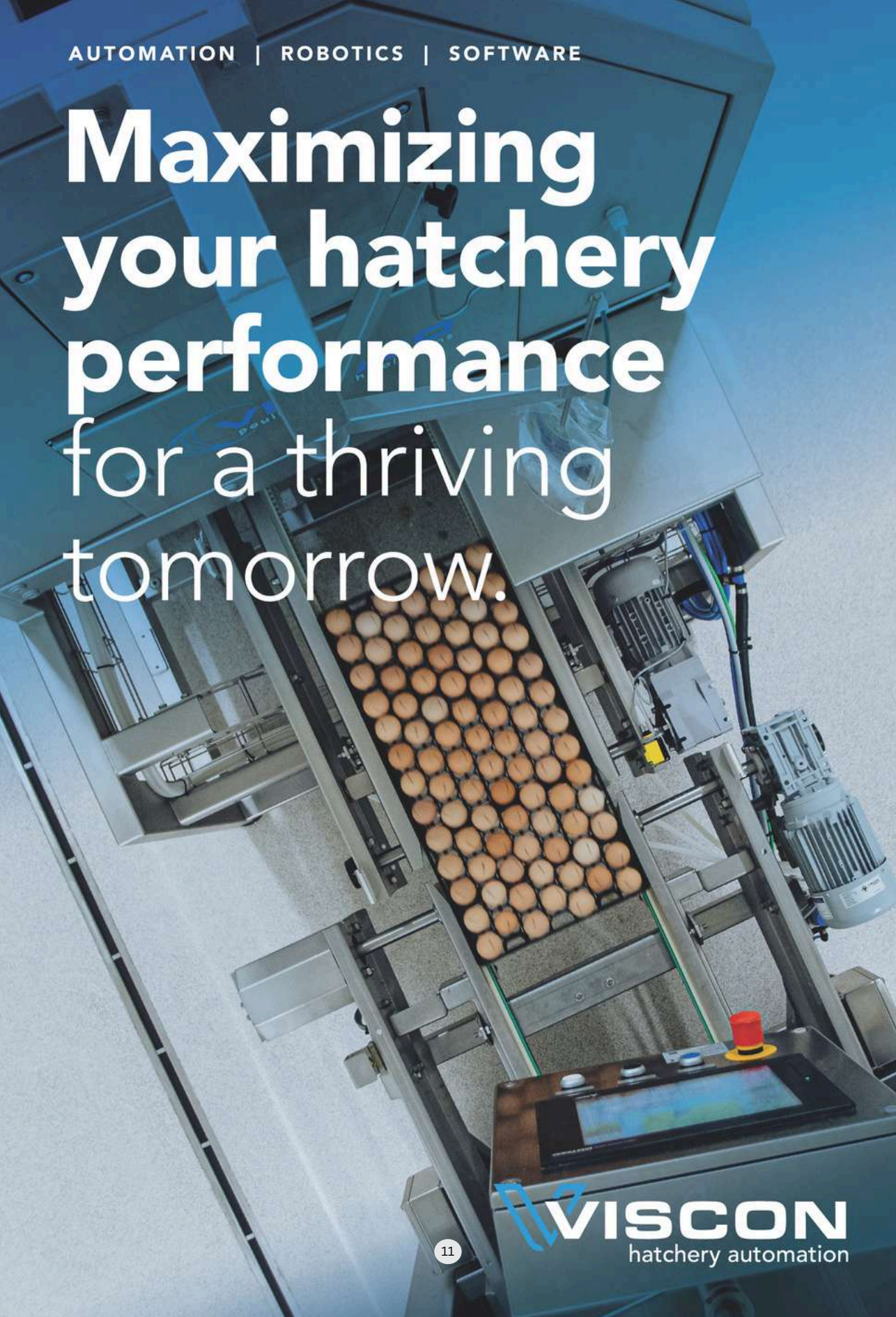
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Thank you to Poultry Performance Plus, Hendrix Genetics, and Ampai Nangsuay for their sponsorship.

2024 IFRG NextGen Funding

Arlette Harder

Arlette Harder (Agricultural engineer with a master's degree in horticultural science) works at the Institute for Agricultural and Urban Ecological Projects (IASP) at the Humboldt-Universität zu Berlin in Germany. Under the supervision of PD Dr. Barbara Tzschentke, she performs incubation studies on mild pre-hatching temperature stimulation of broiler eggs and its long-term effects on health and robustness. Her research work is a focus of the HealthyChick project, which aims to develop innovative methods for minimizing the use of antibiotics in meat-type poultry and is supported by the Federal Ministry of Food and Agriculture, Germany.

2024 IFRG NextGen Funding

Catharina Broekmeulen

My doctoral research, conducted under the supervision of Dr. Michael Toscano and Dr. Sabine Gebhardt-Henrich, focuses on poultry welfare. I have been investigating how various environmental factors during on-farm hatching affect the behavioral development and stress responses of laying hen chicks.

As part of my doctoral studies, I have been an active member of the ChickenStress European Training Network, which has provided opportunities for collaboration with leading labs in endocrinology and neurobiology. Additionally, I have gained expertise in adaptation physiology, animal welfare, behavior, and cognition over the years.

2024 IFRG NextGen Funding

Skarlet Napierkowsk

The production and use of pesticides have significantly increased worldwide, leading to harmful effects on nontarget organisms like poultry. Poultry can be exposed to pesticide residues from feed, even when these residues are within legally permitted levels. The main goal of this project is to assess the impact of three common pesticides tebuconazole, imidacloprid, and glyphosate on rooster semen quality and fertility. The project will monitor pesticide levels in chicken feed, semen, and blood from farms in Poland and France. Roosters will be fed with pesticide-containing feed to evaluate effects on fertility and testicular functions. In vitro experiments will investigate how these pesticides affect sperm. The results may prompt a reevaluation of current pesticide residue limits in poultry feed.

2024 IFRG meeting; Thursday, October 3rd

08:00-09:00	**Registration at meeting room Turgut Albayrak**
09:00-09:15	**Welcome and introduction to the program by IFRG chair (Ampai Nangsuay)**
	Session 1: Fertility (chair - Roos Molenaar)
09:15-10:00	**Keynote lecture; Rooster Fertility: Diagnosis and Conservation** **Anaïs Vitorino Carvalho**, INRAE, France
10:00 -10:20	**Chemerin Present in Egg White, Oviduct and in Embryonic Annexes During the Embryo Development in Hens: a Potential Tool for the Genetic Selection?** **Ophélie Bernardi**, INRAE, France
10:20-10:40	**Plumage Colour Variety Effects on Body Weight and Semen Quality in Leghorn Roosters (Gallus gallus domesticus)** **Marelli Stefano Paolo**, University of Milan, Italy
10:40-11:00	**Coffee/Tea**
	Session 2: Presentations for Nick French Award (chair - Serdar Özlü)
11:00-11:20	**Effects of SPIDES and Preincubation Warming Profile on Embryonic Mortality and Hatchability of Long-Stored Eggs from Young Broiler Grandparent Flocks** **Orhun Tikit,** Aviagen Anadolu, Türkiye
11:20-11:40	**Does Vaccination Cause Stress? Comparison Between the Effects of in ovo and Post-Hatching Vaccination on Stress Level in Chicks** **Weronika Skrypoczko,** Nicolaus Copernicus University in Torun, Poland
11:40-12:00	**The Effects of Light During Incubation and a Post-Hatch Enrichment on White Leghorn Layer Chick Development and Behavior** **Louisa Kosin,** University of Edinburgh, UK
12:00-13:00	**Lunch**
	(Cont.) Session 2: Presentations for Nick French Award (chair - Serdar Özlü)
13:00-13:20	**Influence of Light Exposure and Early Feed Access on the Multitasking Ability in Laying Hen Chicks** **Catharina M.H. Broekmeulen**, University of Bern, Switzerland
13:20-13:40	**The Impact of Pesticides on Rooster Semen Parameters and Hormone Levels During Feeding and After a 4-Week Break** **Skarlet Napierkowska,** Wroclaw University of Environmental and Life Science, Poland
13:40 -14:00	**Effect of Nestmat Hygiene on Hatching Egg Quality and Chick Quality** **Katharina Geers,** University of Applied Sciences Osnabrück, Germany
14:00-14:20	**Morphological Embryo Development During Warming of Broiler Eggs from Storage to Incubation Temperature** **Anne Pennings,** Wageningen University & Research, The Netherlands
14:20-14:50	**Coffee/Tea**
	Session 3: Egg Treatment (chair – Hilke Willemsen)
14:50-15:35	**Keynote lecture: SPIDES - Turning Science into Practice** **Dinah Nicholson**, Aviagen, USA (retired)
15:35-15:55	**The Effects of SPIDES on Hatching and Chick Quality Traits in Different Poultry Species** **Kadir Erensoy,** Ondokuz Mayıs University, Türkiye
15:55-16:15	**How to Warm Eggs from Storage to Incubation Temperature?** **Jan Wijnen,** HatchTech Group, The Netherlands
16:15-16:35	**The Effect of a '24-day Incubation Principle' on Broiler Performance** **Jan Wijnen,** HatchTech Group, The Netherlands
16:35-16:50	**IFRG's Moment of Appreciation**
19:00	**IFRG Barbeque Dinner**

2024 IFRG meeting; Friday, October 4th

	Session 4: Incubation (chair – Miriam Meijerhof)
08:30-09:15	**Keynote lecture: Impact of Incubation Temperature on Meat Quality in Broilers** **Servet Yalçın,** Ege University, Türkiye
09:15-09:35	**Incubator Temperature versus Eggshell Temperature during Artificial Hatching of Ostrich Eggs** **Zanell Brand,** Oudtshoorn Research Farm, South Africa
09:35-09:55	**Effects of Thermal Manipulation of Broiler Embryos from 7 to 16 Days of Incubation on Later Life Thermotolerance** **Itallo Conrado Sousa de Araújo,** Federal University of Minas Gerais, Brazil
09:55-10:15	**Mild Pre-Hatching Temperature Stimulation Improved Post-Hatching Performance in Male and Female Cobb500 Broiler Chickens** **Arlette Harder,** IASP at Humboldt-Universität zu Berlin, Germany
10:15-10:35	**Coffee/Tea**
	Session 5: Incubation (chair - Anne Pennings)
10:35-10:55	**Long-Term Effect on Hypothalamic Plasticity in Chickens Induced by Prenatal Temperature Stimulation Depends on Seasonal Environmental Conditions** **Barbara Tzschentke,** IASP at Humboldt-Universität zu Berlin, Germany
10:55-11:15	**Effect of the Pipping Rate and Hatching Nature on the Development of Artificially Incubated Ostrich Chicks** **Madeli Brand,** Oudtshoorn Research Farm, South Africa
11:15-11:35	**Thermal Imaging of the Temperature of Duck Egg Shells During Incubation in a Prototype Hatching Apparatus with an Automatic Sprinkling System** **Agnieszka Lisowska-Lis,** University of Applied Sciences in Tarnow, Poland
11:35-11:55	**Effect of Egg Turning Completing Time during Incubation on Embryonic Mortality and Hatchability of Broiler Hatching Eggs** **Rana Dişa**, Beypiliç, Türkiye
11:55-13:00	**Lunch**
	Session 6: Incubation and Data Analysis (chair - Orhun Tikit)
13:00-13:20	**Avian Twins** **Marcin W. Lis,** University of Agriculture in Krakow, Poland
13:20-13:40	**Changes of Pekin Duck Hatchability During the Year** **Aleksandra Januszewska,** University of Agriculture in Krakow, Poland
13:40-14:00	**Pullet Hatchability and Quality in Hy-Line Brown Laying Lines: a Data Analysis** **Edgar O. Oviedo-Rondón,** North Carolina State University, USA
14:00-14:20	**Data Analysis of Leghorn Breeder Pullet Hatchability and Quality** **Edgar O. Oviedo-Rondón,** North Carolina State University, USA
14:20-14:40	**Interpretation of Hatchery Breakout Data** **Ron Meijerhof**, Poultry Performance Plus, The Netherlands
14:40:15:00	**Coffee/Tea**
	Session 7: Management and Connecting the Dots (chair – Barbara Tzschentke)
15:00-15:20	**Effect of Broiler Breeder Female Stocking Density during the Laying Period on Egg Production, Mortality, and Hatchability** **Okan Elibol,** Ankara University, Türkiye
15:20-15:40	**Hatchability and First Week Mortality were Severely Impaired After Inoculation of 18-day-Incubated Embryonated Broiler Eggs with Escherichia coli and Enterococcus faecalis** **Thijs Teng Mathieu Manders,** Utrecht University, Netherlands
15:40-16:00	**Understanding the Role of Embryo Development in *in ovo* Vaccination** **Sergio Mesa Raya,** HIPRA Animal Health, Spain
16:00-16:25	**Connecting the dots!** **Michael Wineland,** Hatchery Consult LLC, USA
16:25-16:45	**IFRG Business and closing** (IFRG NextGen Funding, IFRG new chair, 2025 meeting, others)
19:00	**Dinner**

Session 1: Fertility
(Chair - Roos Molenaar)

Rooster Fertility: Diagnosis and Conservation

Anaïs Vitorino Carvalho*

INRAE, PRC, ICF, Université de Tours, PRC, France
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In poultry, far fewer males than females are involved in reproductive management programs. As a result, male selection has important consequences, particularly in terms of economic prospects and dissemination of genetic progress, depending on their reproductive capacity. Developing effective approaches for diagnosing male fertility and maintaining it over long periods of time are therefore two major challenges to improve the poultry industry.

Currently, the assessment of male fertility is based on the in vitro evaluation of semen quality, including semen volume as well as sperm concentration, morphology and motility. However, these criteria correlate poorly with male fertility observed in vivo. In our laboratory, we have developed a new strategy to diagnose sperm fertility based on proteomic methods, essentially Intact Cell MALDI-TOF Mass Spectrometry (ICM-MS), a method that can be applied directly to an isolated cell population and that is capable of rapidly characterizing the intact endogenous peptides and proteins involved in various cellular functions. We demonstrated that ICM-MS applied to ejaculated sperm discriminates chickens according to their fertility status by comparing their semen protein profiles and could be used as a fertility diagnostic test based on predictive mathematical models in an experimental context.

At the same time, biotechnological methods for preserving male fertility are mostly based on sperm cryopreservation. This approach is one of the most widely used to conserve animal genetic resources, based on the collection of large quantities of cells, respect for animal welfare and the possibility of long-term storage and long-distance exchange. While several protocols have been proposed for cryoprotection of chicken spermatozoa, the use of glycerol as cryoprotectant in combination with straw packaging remains the most efficient approach for freezing chicken spermatozoa. However, the presence of glycerol in post-thawed semen samples causes a severe reduction in fertility, leading to the need to remove glycerol prior to insemination. Our recent work has described a new solution to remove glycerol from post-thawed chicken semen. This new method can be processed at room temperature, restores sperm fertility in vivo and can save 44% of the time compared to the classical removal procedure.

All these aspects of male fertility management will be illustrated in my talk.

Keywords: fertility, rooster, cryopreservation, sperm, diagnosis

Chemerin Present in Egg White, Oviduct and in Embryonic Annexes During the Embryo Development in Hens: a Potential Tool for the Genetic Selection?

Ophélie Bernardi[1,2*], Maxime Reverchon[1], Christelle Ramé[2] and Joëlle Dupont[2]

[1] SYSAAF-Syndicat des Sélectionneurs Avicoles et Aquacoles Français, Centre INRAE UMR BOA, F-37380 Nouzilly, France
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One of the goals of breeding companies is the production of viable and robust chicks. New fertility biomarkers, such as chemerin protein, may be used to improve reproductive performances in genetic program and reduce early embryonic mortality.
The aims were to (1) determine chemerin concentrations in albumen during the cycle of laying of broiler and layer hens (2) identify a potential link between chemerin concentrations in albumen and reproductive performances and (3) investigate the presence of chemerin in reproductive tract and its role in embryonic mortality.

Eggs from 100 layer and broiler hens were collected during five consecutive days at three periods: before and after the laying peak, and at the end of laying. For each egg, chemerin concentration in albumen was measured by ELISA assay. Chemerin expression in the magnum was measured by RT-qPCR. Moreover, 80 eggs were incubated to determine the chemerin expression in embryonic annexes. And, 1,200 eggs were injected with chicken chemerin antibodies (0.01, 0.1 and 1 µg/mL) at embryonic day 7 of incubation to evaluate the effect on embryonic mortality.

Both breeds differed in chemerin albumen levels during their laying cycle. Chemerin amounts in albumen were positively correlated with fertility rates for layer hens ($r= 0.26$; $p= 0.01$) and negatively correlated with laying ($r= -0.51$; $p< 0.0001$), fertility ($r= -0.31$; $p= 0.03$) and hatchability ($r= -0.29$; $p= 0.01$) rates for broiler hens. Chemerin expression was higher in the magnum of layer hens compared to broiler hens. During incubation, chemerin levels in allantoic fluid were unchanged whereas significantly increased in amniotic fluid for both strains. The inhibition of chemerin increased embryo mortality from the low dose 0.01 µg/mL antibodies for both strains ($p< 0.0001$).

The concentration of chemerin in albumen fluctuated during the cycle of laying and between breeds. This biomarker was correlated with different reproductive parameters depending of the breed. The inhibition of chemerin in egg white lead to an increase of embryo mortality suggesting a major role of chemerin in embryonic annexes for embryo growth. The use of this new biomarker could improve reproduction rates and subsequently, contribute to economic benefits for breeding companies.

Keywords: chemerin, egg white, embryo, chicken, genetic selection

Plumage Colour Variety Effects on Body Weight and Semen Quality in Leghorn Roosters (*Gallus gallus domesticus*)

Marelli Stefano Paolo[1*], Di Iorio Michele[2], Zaniboni Luisa[1], Perricone Vera[1], Marzoni Margherita[3], Castellini Cesare[4], Iaffaldano Nicolaia[2] and Cerolini Silvia[1]

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[2] Dipartimento Agricoltura, Ambiente e Alimenti, Universita degli Studi del Molise, Campobasso, Italia;
[3] Dipartimento di Scienze Veterinarie, Universita-di Pisa, Pisa, Italia;
[4] Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Universita degli Studi di Perugia, Perugia, Italia
*** Corresponding author:** stefano.marelli@unimi.it

The aim of this study was to point out the differences between Leghorn colour varieties in order to investigate colour-based uniqueness and commonalities in a world-wide known heritage Italian chicken breed. Semen samples collected from 50 Leghorn roosters (N=9 Silver, LHS; N=21 White LHW; N=14 Black, LHB; N=6 Gold, LHG) were analyzed. Roosters (10 months old) were reared on floor following standard chicken breeder management and semen was routinely collected by the dorso-abdominal massage technique. Individual body weight (LBW) was recorded. Three quantitative (Volume VOL; Volume/LBW ratio VWR; Concentration CON) and 11 qualitative parameters (Viability VIT; Total Motility MOT; Progressive Motility PRM; Curvilinear Velocity VCL; Straight line Velocity VSL; Average Path Velocity VAP; Linearity LIN; Straightness STR; Wobble WOB; Amplitude of Lateral Head Displacement ALH, Beat Cross frequency BCF) were analyzed on fresh ejaculates just after collection. ANOVA analysis was carried out using GLM procedure of SAS® 9.4 (colour variety=source of variation; Student's t-test; LSMeans±SE). PCA analysis was performed using Past 4.0 statistic software. Significant differences ($p \leq 0.05$) were found in LBW, VWR, LIN, WOB, ALH. In PCA analysis, LBW describes more than 99% of the variance, VCL and LIN are the qualitative parameters which better describe samples variation. LHG were the heaviest and LHW the lightest roosters (2.51±0.12 vs. 1.91±0.06 Kg), inverse proportion with LBW was recorded in VWR (LHW=0.07±0.01 vs. LHG=0.05±0.01, mL/Kg). LIN (%) and WOB (%) were higher in LHB samples and lower in LHG samples (44.15±1.96 vs. 32.63±3.00; 65.37±1.55 vs. 56.61±2.36). On the contrary, LHG samples showed the highest values for ALH (mm) and LHB the lowest: 4.05±0.23 vs. 3.07±0.15. PCA analysis reveals the differentiating effect of body weight and at the same time the effect of VCL quality parameter as second component. Different levels of clustering ability of the varieties on the two components have been defined. Deep knowledge of breed/variety-specific features under productive, reproductive, and behavioral aspects supplies objective data for biodiversity conservation projects.

Keywords: semen quality, semen phenotype, biodiversity, Italian poultry breeds, animal model

Session 2:

Presentations for Nick French Award
(Chair - Serdar Özlü)

Effects of SPIDES and Preincubation Warming Profile on Embryonic Mortality and Hatchability of Long-Stored Eggs from Young Broiler Grandparent Flocks

Orhun Tikit[1*], Serdar Özlü[2] and Okan Elibol[2]

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Longer egg storage times (>7 d) are common in broiler parent and grandparent hatcheries to obtain the requested flock size. However, prolonged storage is known to decrease hatchability. This study aimed to examine the interaction of short period incubation during egg storage (SPIDES) and preincubation warming (PW) profile after storage on embryonic mortality, and hatchability of long stored eggs.

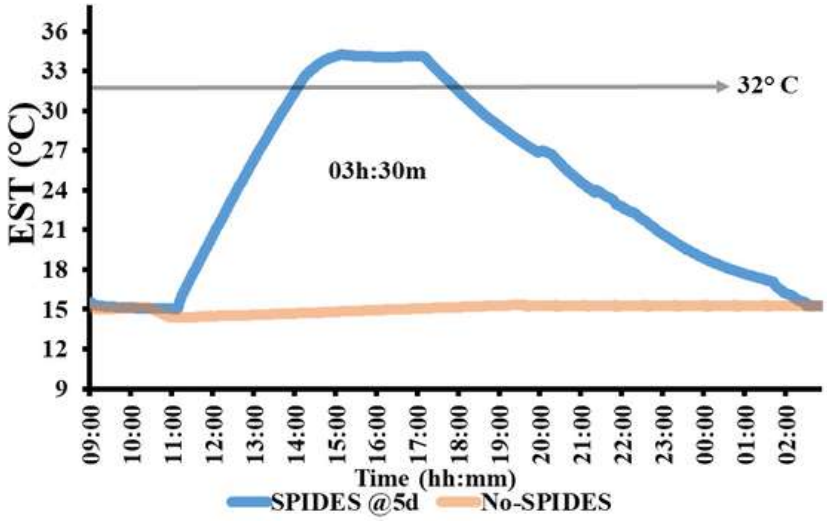
Hatching eggs were obtained from two Ross female line grandparent flocks at 29 and 30 wk of age for trials 1 and 2, respectively. In both trials, 10.800 eggs were stored for 14 d at 15°C. During the storage, the eggs were either kept continuously in the storage room (No-SPIDES) or were subjected to a SPIDES treatment, with 3.5 h above 32°C EST on d 5 of the storage period in a Petersime Re-Store machine (Figure 1). After storage, three preincubation warming profiles were used. These treatments were warming for 6 h (PW6), for 24 h (PW24) at 28°C, or warming eggs from 15°C (storage temperature) to 37.8°C in about 24 h (PWSI24). Preincubation warming procedures were conducted in a Petersime setter before incubation (Figure 2). After all treatments reached to incubation temperature, all eggs in each trial were incubated in the same setter and hatcher. In each trial, a tray of 150 eggs constituted a replicate, and 12 replicate trays (1.800 eggs) were set per subtreatment group. The data from both trials were combined and were subjected to 2-way analysis of variance (ANOVA) with trial as a block using the general linear model (GLM) procedure of SAS.

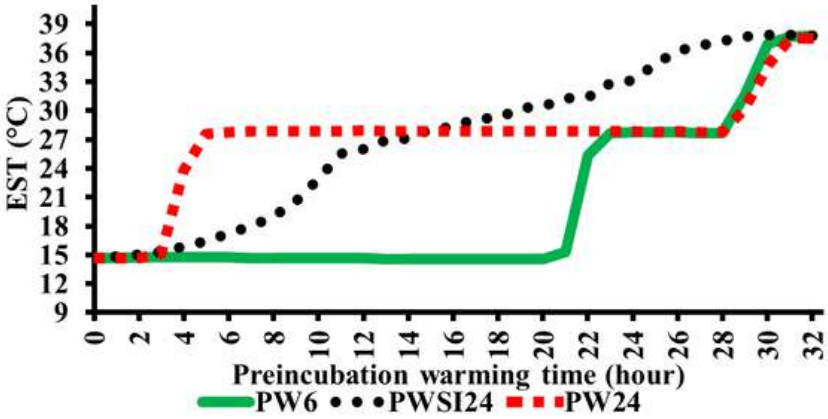
Both SPIDES and long preincubation warming improved the hatchability ($P < 0.05$). However, the interaction between SPIDES and preincubation warming profile was observed for early embryonic mortality ($P = 0.047$) and hatchability of fertile eggs ($P = 0.042$). In No-SPIDES group eggs, hatchability was increased by both longer warming treatments (PW24 and PWSI24) compared with that of 6 h preincubation warming (PW6), due to lower early embryonic mortality, whereas no effect of preincubation warming profile was observed when eggs were subjected to SPIDES at 5 d of 14 d storage period.

We concluded that the detrimental effects of a long storage period may be practically ameliorated by either SPIDES or by longer preincubation warming. However, the positive effect of SPIDES was more evident than the longer preincubation warming for the eggs from young flocks.

Keywords: SPIDES, preincubation warming, embryonic mortality, hatchability

Effects of SPIDES and Preincubation Warming Profile on Embryonic Mortality and Hatchability of Long-Stored Eggs from Young Broiler Grandparent Flocks





Does Vaccination Cause Stress? Comparison Between the Effects of *in ovo* and Post-Hatching Vaccination on Stress Level in Chicks

Weronika Skrypoczko[1,2*], Agnieszka Chłodowska[1], and Anna Sławińska[1]

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Poultry production is one of the largest and fastest-growing sectors of animal husbandry. The significant epizootic pressure increased the demand for poultry vaccinations in the hatcheries. Day-old chicks are routinely vaccinated with the manual subcutaneous injections. The in ovo vaccination is fully automated and performed on eggs, allowing day-old chicks to be transported directly to farms immediately after sorting. The literature confirms that the in ovo vaccination provides earlier immune protection (1) and is less stressful for the chicks (2). The in ovo vaccination eliminates the need for repeated vaccinations at the farm level, and thus reduces the overall stress level (3). The goal of this study is to compare the effects of the in ovo vs. subcutaneous vaccination on the short-term stress in the embryos or day-old chicks. The experiment was conducted on the same batch of the hatching eggs for in ovo and subcutaneous vaccination. On day 18 of the incubation the embryos were vaccinated in ovo using Egginject® (Ceva Ecat-iD). The samples were collected: blood (n=24) for glucose and corticosteroid level and pituitary gland (n=8) for the gene expression. For subcutaneous vaccination, the day-old chicks were vaccinated with Desvac Dovac® (Ceva), and the sampling was repeated. The corticosteroid is a stress hormone and biomarker for short-term stress. It was detected in the blood serum with two methods: ELISA and LC/MS. The gene expression study was based on RNA isolated from pituitary gland and it included genes responsible for activating hypothalamus-pituitary-adrenal axis (6, 7). The glucose level was significantly higher in embryos vaccinated in ovo (196 mg/dL) vs. unvaccinated (161 mg/dL) ($P<0.05$). But, it was the same (about 188 mg/dL) in day-old chicks irrespective of the vaccination ($P>0.05$). The results of the corticosteroid levels and gene expression will be presented. This research contributes to the knowledge on the chicken welfare, stress responses, and immunology.

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Keywords: *in ovo* vaccination, subcutaneous vaccination, short-term stress, chicken welfare, corticosteroid level

The Effects of Light During Incubation and a Post-Hatch Enrichment on White Leghorn Layer Chick Development and Behavior

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Light exposure during artificial poultry egg incubation could be a key element underlying embryo development, post-hatch performance, and post-hatch behavior. While previous research has primarily focused on mitigating negative effects, the influence of light during incubation in relation to positive animal welfare indicators and interaction with enrichment has received less attention. Here, we investigated the effect of light during incubation and environmental enrichment on body mass and chick behavioral responses. We quantified behavioral time budgets and activity levels, environmental usage, and interactions with the enrichment of a dark shelter. White Leghorn chicken eggs were incubated in temperature controlled photoperiodic boxes under either constant full spectrum white light (n=72; 24L:0D) or darkness (n=72; 0L:24D). The chicks were split into 8 pens across 2 experimental rooms post-hatch, and two pens per room contained a dark shelter. Body mass and behavioral data were analyzed with analysis of variance (ANOVA) using the statistical software program R version 4.2.2. Results indicate that at 4 weeks old, chicks incubated under light were heavier compared to those incubated under darkness ($p<0.05$). The dark shelter enrichment did not have an effect on chick body mass development. Light during incubation had no effect on behavioral time budgets and activity levels, but the presence of the dark shelter significantly lowered foraging ($p=0.01$), decreased eating ($p=0.01$), and increased resting ($p<0.001$) behavior. Together these results suggest that light during incubation can affect chick growth rates and this might be a consequence of alternated metabolic and physiologic processes. Data on the dark shelter showed that light during incubation does not influence how chicks would interact with the enrichment. However, chicks use this enrichment, resulting in lower activity levels overall. These findings have the potential to synchronize behavioral patterns and therefore mitigate behavioral problems like feather pecking. The implementation of adequate light-dark cycles into commercial practice is not a huge cost, but it could have a tremendous impact on the welfare of billions of chicks. Further research into the effects of environmental conditions in early life could improve chick health as well as enhance laying hen welfare in later life.

Keywords: light, incubation, hatchability, behavior, dark shelter

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Influence of Light Exposure and Early Feed Access on the Multitasking Ability in Laying Hen Chicks

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On-farm hatching is expected to become available for layers with the ongoing developments regarding in-ovo sexing, although influences of light exposure combined with early feeding during on-farm hatching on behavioral and cognitive development are still poorly understood. Therefore, we investigated effects of continuous light exposure from 18-21 days of incubation and early feeding by assessing multitasking abilities in a 2x2-factorial design with two trials, and the following factors: 1) feed and water (FW), and 2) lighted-incubation (L). Hatching eggs (n=1,280) were exposed to one of the following treatments (4 pens/treatment): FW+L, L-only, FW-only, or deprived. It was hypothesized that FW+L and L-only chicks are better at multitasking than FW-only and deprived chicks. Per pen, focal and companion chicks were selected for the multitasking test and habituated to the arena from 3-9 days of age (DOA). During testing, each duo (n=4/pen) had to forage while predator silhouettes were presented overhead at 10, 11, 12, or 13 DOA. Latency to spot the predator (PRED) and to return to pecking (PECK) were recorded in seconds.

Data were analyzed using generalized mixed-effects models with treatment as fixed factor, and pen nested in trial as random term. The model estimate output was transformed to percentages from odds ratios. Treatment affected PRED and PECK (P=0.002; P=0.012, respectively). FW+L chicks were more likely to have shorter PREDs compared to FW-only and deprived chicks (60%, P=0.007; 59%, P=0.012, respectively). L-only chicks were more likely to have shorter PREDs compared FW-only and deprived chicks (143%, P=0.012; 71%, P=0.018, respectively) indicating that light-incubated chicks were more vigilant, as they noticed sudden changes in their environment faster than dark-incubated chicks did.

FW+L chicks had shorter PECKs compared to FW-only and deprived chicks (61%, P=0.004; 64%, P=0.009, respectively), indicating that FW+L chicks were better at foraging while monitoring for predators simultaneously. In conclusion, shorter latencies suggest that FW+L and L-only chicks might have higher behavioral flexibility and are not as easily distracted or distressed as FW-only and deprived chicks. In a commercial setting, these traits might aid chicks in better adapting to stressful aspects of their daily environment and thereby improve welfare.

Keywords: lateralization, cognition, perinatal environment, hatching system factors

The Impact of Pesticides on Rooster Semen Parameters and Hormone Levels During Feeding and after a 4-Week Break

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This study assessed the effects of pesticides (tebuconazole - TEB, imidacloprid - IMI, glyphosate - GLP) and their mixtures, below MRL levels for feed grain, on rooster semen parameters. It also examined pesticide levels in testes and blood and hormone levels (progesterone, estradiol, testosterone) during exposure and after a 4-week break.
80 Greenlegged Partridge roosters were divided into eight groups: control, TEB, IMI, GLP, TEB+IMI, TEB+GLP, IMI+GLP, and TEB+IMI+GLP, with 10 roosters per group. In Phase I, roosters were with pesticides not exceeding MRL for each grain for 6 weeks, with semen collected twice a week. After 6 weeks, all roosters received control feed without pesticides for 4 weeks. In Phase II, semen was collected again in the 11th week. Semen parameters were analyzed using CASA for motility and flow cytometry for membrane integrity, mitochondrial activity, lipid peroxidation, and apoptosis markers. Pesticide levels in blood serum and testes were measured by liquid chromatography-mass spectrometry, and hormone levels were determined using the RIA method.

Pesticide exposure led to significant differences in semen motility parameters, including average path velocity (VAP), curvilinear velocity (VCL), and straight line velocity (VSL) ($P < 0.05$). The TEB+IMI+GLP group exhibited the highest values, indicating pesticide effects. The percentage of dead cells with lipid peroxidation (LPO) was higher in pesticide-fed groups ($P < 0.05$). Progesterone levels were lower in the TEB, GLP, TEB+IMI, TEB+GLP, IMI+GLP, and TEB+IMI+GLP groups ($P < 0.05$). Estradiol levels showed no significant differences ($P > 0.05$), while testosterone levels were reduced in the TEB+IMI and IMI+GLP groups ($P < 0.05$). After the 4-week break, no significant differences in semen parameters were observed between groups ($P > 0.05$), except for decreased VAP and VSL in the IMI+GLP group, indicating some reversibility. Pesticide levels in serum and testes decreased during the break, reflecting reduced exposure.Pesticide exposure significantly impacted sperm parameters and hormone levels, increasing dead cells with lipid peroxidation and reducing testosterone and progesterone levels. Semen motility was highest in the group exposed to all three pesticides. However, after a 4-week break, semen parameters normalized, and pesticide levels significantly decreased, suggesting reversible effects of pesticide exposure.

Keywords: pesticides, semen, progesterone, testosterone, testes

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Effect of Nestmat Hygiene on Hatching Egg Quality and Chick Quality

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Good hatching egg quality describes visible clean nest eggs without damages like cracks and deviations. The hygiene of hatching eggs like bacterial load, might also affect chick-quality. To evaluate the effectiveness of two different nest hygiene procedures, a trial was carried out in the nests of ROSS 308 grandparent flocks under commercial conditions.

Two nest hygiene procedures were carried out each in one male line and one female line. The houses for the trials had two nestrows: one treatment- and one control row. One trial involved the use of disinfection powder Dry Care Des (powder treatment), which was spread into the nests three times a week with a dosage of 75 g/m² for each application. The other procedure involved a change of nestmats in production weeks 38 and 48 (nestmat change treatment). For each nestrow in each house, the total bacterial count (TBC) of 5 eggs, the number of eggs of category 1 (clean nest eggs), category 2 (mildly soiled nest eggs), and non-hatching eggs (all other eggs), and break-out data of 750 eggs was recorded every two weeks. The collected data of the hatching eggs was not statistically analysed, however the break-out results showed numerically lower values for both of the treatment groups compared to the control groups.

A Field Mini Pen trial (FiMiPeT), was carried out twice, to investigate whether the two different hygiene procedures had an effect on first-week mortality (FWM). For the two FiMiPeT's, the by-products from the female line were placed at a broiler farm in 20 randomized mini pens with 180 chicks each. The FWM per group was recorded and significant differences were analysed using the Wilcoxon rank test with $p = 0.05$.

The results showed no significant effect on FWM for hygiene procedures. However, in both the FiMiPeT's, the powder treatment-group, and the nestmat change-group, showed very low FWM (up to 20% lower) compared to average FWM for commercial deliveries. This might suggest that both the treatments might reduce the bacterial load on the hatching eggs, subsequently reducing FWM. However, further research is required to support this hypothesis.

Keywords: hatching egg quality, bacterial load, chick quality, first-week mortality, FiMiPeT

Morphological Embryo Development During Warming of Broiler Eggs from Storage to Incubation Temperature

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Between oviposition and the onset of incubation (eggshell temperature (EST) = 37.8°C), chicken embryo development is affected by various external factors. Especially temperature plays an important role, both during egg storage and during warming of the eggs from storage temperature to incubation temperature ('pre-warming'). The rate and duration of pre-warming are expected to affect the rate of morphological embryo development, and consequently, total incubation duration. When eggs are exposed to a prolonged pre-warming period, with a slower rate of warming, embryos are allowed more time to develop already before the onset of incubation, which may shorten the incubation period itself. However, it is unknown how morphological embryo development is influenced by different pre-warming durations, and whether this interacts with egg storage duration. The objective of this study was to investigate morphological development during different pre-warming profiles and in the subsequent incubation period. A total of 14,400 Ross 308 eggs, originating from one single breeder flock (aged 37-45 weeks), were used in a 2x3 factorial experiment with four consecutive batches to study the effects of egg storage (4 and 14 days) and pre-warming (10, 24, and 144 hours). After storage, eggs were pre-warmed from storage temperature (≈18°C EST) to 29.4°C EST in 5 hours for all treatments, and from 29.4°C to incubation temperature (37.8°C EST) within the remaining 5, 19, or 139 hours. After pre-warming, EST was maintained at 37.8°C throughout the remaining incubation period. Embryos (n = 630) were isolated using the filter ring technique (Gupta & Bakst, 1993) and staged (Eyal-Giladi & Kochav, 1976; Hamburger & Hamilton, 1951) on 5 time points: day of lay, after egg storage/just prior to the start of pre-warming, halfway through pre-warming, at the end of end of pre-warming, and 3 days after pre-warming. Embryo weight was measured every 3 days of incubation until transfer on day 18. To determine the hatch window, hatchability, and total incubation duration, all hatcher baskets were checked every 8 hours during the hatching phase for newly hatched chicks. The data still has to be analyzed, and results will be presented during the conference.

Keywords: embryo development, morphology, pre-warming, egg storage, incubation duration

Session 3: Egg Treatment (Chair – Hilke Willemsen)

SPIDES - Turning Science into Practice

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Research into the effects of delivering short periods at incubation temperature during egg storage (SPIDES), was first reported by Jackson (1912), who warmed eggs under a broody hen. Kosin (1956), Kan et al. (1962), Meir and Ar (1998) and Ar and Meir (2002) in trials using incubators, all reported results where short periods of heating, repeated once or twice a day for 1–5 h gave some hatchability improvement.

Despite consistent experimental results, by 2009 very few commercial hatcheries heat treated eggs during storage. The rest saw it as an unnecessary complication, had not seen any benefit, or would not allow temperatures to fluctuate during storage. Nonetheless, some hatcheries need to incubate stored eggs, forfeiting chicks and needing complicated setting patterns to allow for delayed embryo development. In 2011 Aviagen started exploring combinations of treatment duration and treatment frequency. We found that in commercial incubators, heating and cooling times varied widely depending on incubator type and design, which is why following exposure times from laboratory experiments using small-scale incubators was not helpful.

Initial results, reported at the IFRG in 2011, showed promise and in 2013 we reported results (Nicholson et al. 2013). These showed the procedure to be robust, provided that the cumulative time egg shell temperature (EST) was above 32oC did not exceed 15 hours. SPIDES treatment will recover 60-70% of the hatchability lost due to egg age, reduce the hatch delay in old eggs and reduce culling levels at day old.

Later experiments with collaborators in various research labs showed that SPIDES and egg turning had an additive effect, suggesting different mechanisms (Özlü et al. 2021) and that SPIDES limited the damage to gene transcription pathways seen in embryos in old eggs (Bakst et al. 2016, Brady et al. 2022).

Since 2011, there have been 35 published reports about SPIDES trials, some looking at different species and other, more recent ones reporting a positive impact on broiler performance. Companies designing commercial incubators all offer ‘SPIDES’ machines, and SPIDES is used not only for high generation stock, but also by many hatcheries producing commercial broiler chicks.

Keywords: SPIDES, egg Storage, hatchability, hatch time, culls

The Effects of SPIDES on Hatching and Chick Quality Traits in Different Poultry Species

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The aim of this study was to determine the effects of SPIDES (short period of incubation during egg storage) application on hatching traits (hatching results, egg weight loss, hatching time) and chick quality (Tona score) in different poultry species. A total of 3000 hatching eggs (600 of each species in 10 replications) from quail (Pharaoh), Guinea fowl (Pearl Grey), goose (Turkish), partridge (Rock) and chicken (Atak-S) species were used. Eggs were stored at 17 ºC and 75% Rh conditions for 10 days (Control "C") prior to incubation. The random half of these eggs were exposed to 35 °C for 4 h at 5 days of storage and then continued storage at 17 °C (SPIDES "S"). Each species was subjected to standard incubation conditions in separate machines. There was no difference between C and S groups for egg weights at the beginning of incubation ($P>0.05$). The highest egg weight loss was in Guinea fowls and quails, while the lowest was in geese ($P<0.05$). While chickens and quails had the highest fertility, the lowest rate was determined in the partridges ($P<0.01$). There was no difference between C and S groups in terms of fertility ($P>0.05$). Early embryonic mortality was about 4% and late embryonic mortality was about 2% higher in the C group. Therefore, the S group had better hatchability of fertile eggs in all species and was higher than the C group with an overall average of about 6% ($P<0.01$). There was no difference between C and S groups for chick weight, chick length, chick quality score and chick yield ($P>0.05$). The highest chick quality score (99.12%) was determined in quails and the lowest (95.66%) in geese ($P<0.01$). The S group chicks hatched 2 h earlier than C group chicks ($P<0.01$). In conclusion, the differences between the species were significant for almost all traits as expected ($P<0.05$). The SPIDES significantly reduced early embryonic mortality, increased the hatchability of fertile eggs and shortened the incubation period as well. Therefore, the SPIDES is strongly recommended for a better hatching performance when the eggs of these five poultry species are stored for long periods.

Keywords: poultry species, SPIDES, embryonic mortality, hatchability, chick quality.

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How to Warm Eggs from Storage to Incubation Temperature?

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Chicken hatching eggs contain an embryo blastoderm already at moment of lay. In commercial practice, these eggs are stored ≤18°C to preserve their quality until incubation. Once incubation starts, they have to be warmed to 37.8°C eggshell temperature (EST), meaning that the embryo blastoderm undergoes a temperature transition of approximately 20°C. Embryos are poikilotherm and therefore blastoderm development is affected by the rate and duration of this temperature transition. It has been shown that the rate and duration for the transition from storage to 29.4°C EST are of minor importance as long as condensation will be prevented. But from 29.4°C EST onwards, a linear increase during 17 hours to 37.8°C EST lowered early embryo mortality compared to shorter prewarming durations. Until now, durations >17 hours for this specific temperature transition have never been studied. All eggs were linearly warmed in 5 hours from storage temperature to 29.4°C EST whereafter the duration of linearly prewarming them from 29.4°C to 37.8°C EST was increased from 17 hours to 8 days stepwise in 18 consecutive experiments using a total of 146,880 eggs. For each experiment, eggs originated either from broiler (Ross308) or layer (Dekalbwhite) parent flocks of various ages (26-58 wk) and were stored 0 to 23 days prior to incubation. Early embryo mortality and hatchability of set eggs were observed. Results indicated that a duration of 6 days was the most optimal duration of prewarming eggs from 29.4°C to 37.8°C EST. Early embryo mortality was reduced and hatchability was increased between 1.2 to 21.8% in 14 out of 18 experiments (P≤0.04). In conclusion, early embryo mortality is affected by the rate and duration with which eggs are warmed prior to incubation. Very gradual preincubation warming of eggs during 6 days from storage to incubation temperature can be considered as a strategy to enhance hatchability as it reduces the relatively high early embryo mortality that is found during artificial incubation. However, the downside of this strategy is that it prolongs the total incubation duration (start of prewarming until pull) by approximately 3 days compared to the standard practice of ≤0.5 day prewarming.

Keywords: prewarming, hatchability, embryo development, poultry incubation, eggshell temperature

The effect of a '24-day incubation principle' on broiler performance

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Recently HatchTech introduced SetCare® on the market. This setter comprises the gradual warming of chicken eggs during six days from storage- to incubation temperature at high RH and CO2 levels and a total incubation duration of 24 days. In-company studies with SetCare found longer chicks at hatch compared to conventionally incubated chicks. A positive correlation between hatchling length and post hatch performance of broilers has been shown. It was therefore hypothesized that SetCare advances broiler performance. Besides incubation, broiler performance is affected by the moment of first feed and water access: directly after hatch (referred to as 'early feeding') or delayed till arrival in the broiler house. Early feeding seems to be optimal, but effects can depend on chick quality. It can be hypothesized that the improved chick quality realized through SetCare amplifies the positive effects of early feeding on broiler performance. To study this, long stored eggs from a 34 wk Ross308 parent flock were incubated in a 2x2 factorial design in different setter systems (SetCare vs conventional) and hatcher systems (HatchCare vs conventional hatcher with delayed feeding). At hatch, chicks (N=576) of both sexes were equally divided over 32 floor pens. Broilers and feed were bulk weighed weekly per pen and ADFI, ADG, and FCR were calculated. Mortality was observed daily to determine survival probability. No setter x hatcher interaction was found, except for survivability (P=0.02). SetCare × HatchCare had 6.3% higher survivability probability compared to conventional × HatchCare. SetCare resulted in higher BW at all ages (P<0.02) as well as higher ADFI and ADG (P<0.03) and lower FCR (P=0.03) over the total growth period compared to conventional setter. HatchCare resulted in a higher BW at all ages (P<0.01) and tended to increase ADG and ADFI (P<0.08) over the total growth period compared to conventional hatcher (P<0.01), whereas FCR was not different (P=0.26). In experimental conditions, both SetCare and HatchCare seem to benefit broiler performance over conventional systems, but their combination does not have a clear added positive effect and a field study is proposed to verify results in commercial practice.

Keywords: broiler chicken, pre-warming, SetCare incubation, early feeding, growth performance

Session 4: Incubation (Chair – Miriam Meijerhof)

Impact of Incubation Temperature on Meat Quality in Broilers

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The broiler industry plays a key role in supplying protein sources for people as having advantages over red meats in terms of the easily digestible protein source, greater protein/fat ratio, lower cost, shorter production duration, and lower carbon fingerprint. During the last 60 years, the selection program of breeding companies has focused on growth rate, feed efficiency, and breast meat yield to meet consumer demand for chicken meat. This increase in growth rate and breast yield is associated with muscle development and meat quality in commercial chickens. Chicken skeletal muscle development occurs between the beginning of embryonic development and early post-hatch. While the number of muscle fibers is fixed during embryogenesis, postnatal skeletal muscle growth depends mainly on muscle fiber volume. In recent years, muscle development in embryos has gained attention. In chicken embryos, environmental factors in the incubator such as temperature, humidity, ventilation, turning, and lighting determine embryonic development. Within these factors incubation temperature, which is between 37.5 and 37.8°C in modern today's incubators, is the most important factor that optimizes hatchability. However, during the natural nest, embryos face daily fluctuating temperatures. Incubation temperatures higher or lower than optimum may have long-lasting effects on post-hatch growth performance, behavior, and locomotor activity. Previous studies have shown that higher cyclic incubation temperatures affect muscle growth during embryogenesis, increasing muscle relative weight and promoting fiber development. This effect may be mediated by IGF-I gene expression and muscle marker genes (myogenin, MyoD, Pax7) without significant change in breast meat pH, lightness, redness, and drip loss. However, different durations, timing, and temperatures appear to have different effects on muscle growth and meat quality, and its effect on muscle myopathies varies across many studies and the information is conflicting. Further results showed that the response of skeletal muscles to changes in the incubation temperature would differ between species, egg- and meat-type chickens, and commercial strains. Critically, understanding the impact of incubation temperature on muscle development and meat quality can enable the development of new approaches to poultry production.

Keywords: Incubation temperature, muscle growth, meat quality myopathies

Incubator Temperature versus Eggshell Temperature During Artificial Hatching of Ostrich Eggs

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The incubators currently in use for the artificial incubation of ostrich eggs were primarily designed for the chicken incubation industry and thus not designed to hatch the larger ostrich eggs. Problems may arise if incubators do not have sufficient heating, cooling and air exchange for the larger ostrich eggs set. Incubator temperature settings are used during incubation, but due to the size of the developing ostrich embryo, it is vital to investigate eggshell temperature (EST).

The differences between incubator temperature and EST during incubation of ostrich eggs were investigated on the Oudtshoorn Research farm, South Africa, during the 2000 and 2021 breeding seasons. The set temperature for the incubators was 36.4°C. Heat sensors were placed at different locations in the incubators to monitor heat distribution throughout and at the same locations, sensors were placed on the nearest eggs to be able to determine the effect of incubator temperatures on eggshell temperature (EST). The data collected over the incubation period of 42 days, included temperature readings from 71 incubator probes (6334 readings/probe) and between 2878 and 6334 readings from 186 probes attached to eggs for EST.

During the setter phase (≤ 36 days of incubation) significant differences were found between the different sectors in the incubators, ranging from 34.7±0.01°C in the middle sector of the incubator to 35.7±0.01°C at the top sector of the incubator. EST was significantly higher than incubator temperature for each of the corresponding sectors, but the biggest difference was at the middle section of the incubator where the mean EST was 36.0±0.01°C, while the mean incubator temperature was 34.7±0.01°C. There was no significant difference between the overall mean incubator temperature and mean EST up till day 37 of incubation. Between days 37 and 38 of incubation a sharp increase in EST (from 36.4±0.3°C to 37.4±1.1°C) occurred and, while incubator temperature also increased during this period (from 35.2±2.4°C to 35.7±0.3°C), it was significantly lower than of EST. Up to 35 days of incubation, the EST did not differ for eggs that were either infertile, early embryonic (EED) and late embryonic deaths (LED), or produced a live chick.

These results showed that the placement of controller sensors is very important, because depending on the fan placement, heat distribution differs within incubators. The effect of the rapid increase in EST during the hatcher phase (≥ 36 days of incubation) on incubator temperature needs to be investigated further in order to improve management of hatcher temperature during this critical stage of incubation.

Keywords: artificial incubation, embryonic deaths, hatching temperature, heat distribution

Effects of Thermal Manipulation of Broiler Embryos From 7 to 16 Days of Incubation on Later Life Thermotolerance

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An experiment was conducted to investigate five different thermal manipulation (TM) protocols utilizing eggshell temperature (EST). Group 1 was set at 37.5°C and 60% relative humidity (RH) from day 1 to 19 of incubation. Treatments in groups 2 to 5 covered days 7 to 16 of incubation, employing different EST and 65% RH (group 2: 39.5°C/6h/day; group 3: 39.5°C/12h/day; group 4: 40.5°C/6h/day; and group 5: 40.5°C/12h/day). A total of 4,300 eggs were distributed across five different setters with a capacity of 860 eggs each. After hatching, 540 male Ross chicks (108 per treatment) were reared with six pens of 18 chicks each. Until 21 days of age, room temperature followed the Ross guidelines, whereafter, all groups were subjected to heat stress (8h/32°C) from day 21 to 28 post-hatch. Performance and cloacal temperature were evaluated at 28 days of age. Chicks from group 2 (39.5°C/6h/day) had lower cloacal temperature during heat stress ($P<0.05$; 41.0°C) compared to the other groups: 42.9 41.5, 41.5, and 41.1°C for groups 1, 3, 4, and 5, respectively. Body weight gain (BWG) at 28 days of age was lower for group 5 (1,425g), and this group also had the worst feed conversion ratio (FCR) (1.47; $P<0.05$), whereas there was no difference among the other groups (1,574 g and 1.33 respectively on average). Feed intake was not affected ($P>0.05$). Group 1 chicks had a higher mortality rate (10.7%) between days 21 and 28 (immediately after the onset of heat stress) compared to other groups ($P<0.05$). Based on cloacal temperature and mortality rate, it can be concluded that the best TM protocol was 39.5°C EST between days 7 and 16 for 6 hours per day.

Keywords: adaptation, broiler production, cloacal temperature, eggshell temperature, heat stress

Mild Pre-Hatching Temperature Stimulation Improved Post-Hatching Performance in Male and Female Cobb500 Broiler Chickens

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The aim of this study was to investigate the effect of mild pre-hatching temperature stimulation (MTS) of Cobb500 eggs on hatching results as well as on growth performance and organ development on day 7 post-hatching. 355 eggs were incubated under standard incubation conditions (37.3°C, 55% relative humidity) until transfer on day 17 where living embryos were randomly divided in two groups: control (C: standard incubation conditions, n=156) and MTS (+1°C for 2 h per day, on days 17-20, n=156). After hatching, chickens were sorted by sex (feather sexing). Chick quality (Pasgar©Score) and body weight (BW) as well as in 20 birds (10 female/10 male) of each group yolk sac to BW ratio (YBW ratio) were analyzed. In a subsequent growing trial of 7 days, growth performance was measured, and bursa and heart samples were collected and weighed. MTS improved hatching rate (MTS: 90.4%; C:87.2%). As usual, sex ratio of the hatched chicks was characterized by slightly more hatched females and similar in both groups (MTS females: 54.6%, MTS males: 45.4; C females: 55.1, C males: 44.9). MTS did not significantly change chick weight, chick quality and YBW ratio at hatch. YBW ratio was lower than 10% in both groups (MTS: 8.5%; C: 6.9%) but, MTS chickens of both sexes had numerically higher YBW ratio than control chicks. No statistically significant sex differences were found within the groups. During the first seven days post-hatching mortality was zero in all groups. MTS chickens had a statistically significant higher BW (MTS, BW 144.8 g ± 19.2; C, BW 137.8 g ± 17.0, p = 0.01) and body weight gain (BWG) when compared to control chickens and showed a tendency towards improved feed conversion (FC; MTS: 0.98 ± 0.04 kg/kg, C: 1.03 ± 0.03 kg/kg) and feed intake (FI; MTS: 15.53 ± 0.5 g/broiler/d, C: 15.99 ± 0.2 g/broiler/d). There was no significant influence of sex of the birds on all parameters within the groups. But, numerically MTS female chickens showed the highest BW and BWG within the MTS group. No statistically significant differences were found for the relative bursa and heart weights between the groups. As conclusion, MTS has no negative effect on hatching and 7-day growth performance and appears to have the potential to improve hatching rate and 7-day performance, especially BW and BWG, in male and female Cobb500 broilers.

Keywords: Cobb500, temperature stimulation, pre-hatching, yolk sac to body weight ratio, Pasgar©Score

This study was the first part of the project Healthy Chick, supported by the Federal Ministry of Food and Agriculture, Germany.

Session 5: Incubation (Chair - Anne Pennings)

Long-Term Effect on Hypothalamic Plasticity in Chickens Induced by Prenatal Temperature Stimulation Depends on Seasonal Environmental Conditions

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In precocial birds, thermoregulation develops early in ontogeny, and perinatal thermal experiences can affect its long-term functionality. The aim of the study was to elucidate whether these early adaptation processes affect hypothalamic neuronal molecular biomarkers related to developmental plasticity and energy metabolism. We investigated how a short-term prenatal temperature training (PTT, +1°C for 2 hrs) with 126 eggs in total, incubated at 37.3°C, of which 62 were temperature trained on embryonic days 17-20, affects the basal mRNA expression levels of Bdnf, Npy, and Pomc at 35 days of age in the hypothalamic nucleus infundibular (IN) in broiler chickens (ROSS 308). The hypothesis was that PTT establishes long-term changes in basal Bdnf mRNA expression levels reflecting an increased adaptive plasticity, and long-term changes in basal Npy and Pomc mRNA expression levels reflecting shifts of the physiological feed-back cycles underlying feeding behaviour, feed conversion, and satiety. We conducted a 35 days growing trial during winter (November/December) and a summer trial (July/August) with consistently higher ambient temperatures. Mean mRNA expression levels at 35 days of age were descriptively higher for Npy, Pomc, and Bdnf in the PTT group compared to the control group. The group difference for Bdnf was statistically significant when analyzed across both trials (N=72, padjust= 0.048). Further analysis revealed that this effect was mainly driven by differences in the summer trial. For all three target genes descriptively, we observed highest mean mRNA expression levels for the PTT group in the winter trial and lowest expression levels for the control group in the summer trial, with the PTT rescuing the dampening effect of the higher ambient temperature on the expression levels. For Bdnf, the difference was statistically significant even after adjusting for multiple testing. We did not observe any significant sex differences for any of the target genes in both trials. Our findings indicate that short-term PTT during a critical developmental period of the thermoregulatory system induces changes in mRNA in avian hypothalamic neurons that are associated with developmental plasticity and also correspond to the improved performance and robustness parameters found in numerous previous studies. This PTT effect may contribute to a long-term physiological adaptation that can improve resistance to stressful environmental conditions, such as high ambient temperatures, in broiler chickens.

Keywords: thermoregulation, gene expression, energy metabolism, brain-derived neurotrophic factor, broiler chicken

Effect of the Pipping Rate and Hatching Nature on the Development of Artificially Incubated Ostrich Chicks

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High levels of embryonic mortalities are a cause for concern because of its marked effect on cost-effective commercial ostrich farming. The number of viable chicks produced can be boosted by identifying the most plausible protocol to support hatching chicks.

Data for this study were collected from the commercial, pair-bred ostrich flock on the Oudtshoorn Research Farm, South Africa. Data were collected from 169 fertile eggs and divided randomly into three groups on days 41, 42 and 43 of incubation. The treatment groups were: 1) hatchlings that reached climax and broke free from the eggshell by themselves; 2) hatchlings that were assisted to reach climax at the first signs of external pipping; and 3) hatchlings that were removed from the eggshell at the first sign of external pipping. Eggs with hatchlings that pipped internally after 43 days of incubation, but failed to pip externally, were cracked to aid the hatchlings (Treatment 4). Clinical measurements (heart rate, temperature, level of oedema) were recorded on day of hatch, while body weight was recorded seven days after hatch and then on days 28, 84, 147, 227, 300 and 365 of age.

Comparing the different treatments, chicks that were assisted after internal pipping (Treatment 4) took substantially longer to hatch than the other treatments. The heart rate of 115 beats per minute (bpm) for chicks hatching on their own was lower than the 132-bpm recorded for chicks in the other treatment groups. Up to day two after hatching, a decline of ~4% was found in chick weight for all treatment groups. Chick weight increased from 0.85 kg to 1.11 kg, with an increase in age up to seven days. At the age of 147 days a marked difference began to appear between the different treatment groups. Live weight for the chicks hatching on their own was higher at 12.6% and 24.6% respectively, if compared with the chicks where the eggshell was cracked and the chicks where the eggshell was removed after external pipping.

Results from this study clearly demonstrate that chicks benefit from climaxing by themselves. Where possible no intervention should be the preferred mode of action for chicks to achieve this. In the case of chicks struggling to hatch, this study may be used as a guide to inform future hatchery operators on the specific stages where monitoring and assistance are important in those chicks experiencing problems during hatching.

Keywords: chick weight, hatchery management, heart rate, mortality hatchability, oedema.

Thermal Imaging of the Temperature of Duck Egg Shells During Incubation in a Prototype Hatching Apparatus with an Automatic Sprinkling System

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The aim of this study was to investigate the heat emission from Pekin duck eggs from 30- and 50-week-old parental flocks, before and after cooling inside the setter.

Eggs were incubated in a prototype setter (modified Sommen S hatching apparatus; 28,224 eggs, 8 carts, 28 trays, 126 eggs/tray). The incubation program assumed a gradual decrease in machine air temperature (MT) from 38.0°C to 36.8°C and relative humidity from 70% to 60% between the 1st and 28th incubation days (E1-E28). An internal automatic system for cooling (from E10) and sprinkling the eggs (from E16) was tested. The eggshell temperature (EST) was imaged using a FLIR E50 thermal camera (464 × 348 pixels) on days E2, E6, E13, and E26 before and after the cooling cycle. Thermograms were taken inside incubators for the whole cart and outside for individual trays. Thermograms were analyzed with FLIR Tools+ to compare heat emission from the eggs at the same tray and at different levels of the setter.

EST reached MT on E2 and began to exceed it on E6. EST exceeded the MT by (mean±SD) 1.2±0.26°C on E13 and 2.2±0.41°C on E23. The cooling and sprinkling decreased EST on E23 from 39.0±0.41 to 36.7±0.75°C. However, analysis of thermograms revealed that cooling the eggs was uneven and depended on the position of the eggs on the tray and the level of the cart. The stronger-cooled area (EST 36.1±0.51°C) was usually located at the tray's centre, while less-cooled one the outer edge of the tray (EST 37.2±0.30°C). However, the eggs in the upper levels were stronger-cooled closer to the medial edge. On these trays, cooling was a slightly lower but more evenly despite (36.8±0.23°C) compared to the lower ones (35.7±0.83°C).

The modifications of incubation technology, despite some imperfections, resulted in an increase in hatchability year-on-year from 70.0±9.98% to 82.2±6.6%. This indicates that while the internal egg cooling system in the incubator can be effective, the system requires adjustment. Simultaneously, thermography seems to be a useful method for non-invasive monitoring and detection of weak points of the incubation process.

Keywords: thermography, eggshell temperature, waterfowl, eggs cooling

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Effect of Egg Turning Completing Time During Incubation on Embryonic Mortality and Hatchability of Broiler Hatching Eggs

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The effect of turning duration and frequency during incubation has been widely investigated. However, little is known about the effects of turning completing time on hatchability. Furthermore, it is unclear which optimum turning completing time is required to see the benefits of egg turning. In this study, the effects of turning completing time during incubation on fertile hatchability and embryonic mortality were evaluated. Hatching eggs were collected from a 51-week-old commercial flock of Ross 308 broiler breeders and stored for 3 d at 16°C. A total of 7,200 eggs were placed randomly in 3 trolleys (treatments) and set in a commercial incubator (Petersime NV). In this experiment, the turning completing time to one side of each trolley was adjusted individually. The turning completing time was set normal, and turning took less than one minute (<1min = control) for one trolley; the other two trolleys were set for longer completing times that took 30 minutes or 60 minutes to one side. In all trolleys (treatments), eggs were subjected to turning angles of 38° and turned once an hour up to 18.5 d of incubation (transfer time). Therefore, the trolley was turning continuously in the 60 min group. There were 16 trays in each treatment, with 150 eggs constituting a replicate. For each replicate tray, fertile hatchability, embryonic mortalities, malpositioned embryos (embryos with their heads in the small ends of the eggs), and second-grade chick percentages were calculated. The data were subjected to One-Way ANOVA using the general linear model (GLM) procedure of SAS.

Fertile hatchability was decreased (≈2.4%) in 30 and 60-minute turning completing time groups compared to control (<1 minute) due to numerically higher mid and significantly higher malpositioned embryos and late embryonic mortality. These data demonstrated that a longer turning completing time decreased (P = 0.008) hatchability compared to a control during incubation. The possible reason for the lower hatchability in the longer turning completing time is unclear, but presumably, it might be due to longer turning don't have helped adjust the positioning of the embryo to achieve the correct formation to hatch.

Keywords: incubation, turning completing time, embryonic mortality, malposition, hatchability

Session 6: Incubation and Data Analysis (Chair - Orhun Tikit)

Avian Twins

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The phenomenon of multiple embryos in birds and other oviparous vertebrates is rare and poorly studied. Romanoff and Romanoff (1972) believe that avian twins can originate from: 1) double-yolk egg; 2) multiple blastodiscs in one yolk; and 3) disturbances at the gastrulation of a single blastoderm resulting in monovular, monozygotic twins.

The most obvious are dizygotic twins developing in multi-yolks eggs. These eggs are formed by ovulation disturbances, and their structure varies among poultry species. Double-yolk eggs, where yolks touch and are often enclosed by a shared vitelline membrane (type A by the classification of Romanoff and Romanoff, 1949), predominate in waterfowl, while separated yolks (types B and C) are usually found in chickens. Hatchability from double-yolk eggs is very low (up to 10%), affecting only eggs in which a single chick develops. This is despite the fact that both oocytes are usually fertilized and twins can survive even to the hatching stage.

A more intriguing phenomenon is presented by monovular twin embryos (MTE). In our study, 13 MTEs (0.66%) were found in 1,955 embryonated eggs from 20 pedigree breeding flocks of the zatorska goose. Nearly half of the MTEs developed properly until hatching. Hatching was impeded by the need to share a common yolk sac and the positioning of embryos, which prevented one gosling from pipping and breathing. Aside from one case of conjugation, no other malformations were observed. Genotyping of microsatellite loci indicated their development from a single blastoderm (except in one case where development from multiple blastodiscs was possible). Pedigree analysis did not show a genetic predisposition to twinning. On the other hand, the incidence of MTE increased with egg storage time and was 0.28 and 1.46% of embryonated eggs for 2–8 days and 9–15 storing days, respectively. Pokhrel et al. (2018) found that dramatic cytoarchitectural changes in blastomeres occur during prolonged egg storage, which can result in body duplication (polydactyly, conjoined twins). Additionally, the development of MTEs seems to be stimulated by subjecting embryos to hypothermia (overcooling) before incubation, usually related to the season and atmospheric conditions (Batt et al., 1975).

Our results support the hypothesis that MTEs development is stimulated more by environmental conditions than genetic factors.

Keywords: poultry, monovular twin embryos; disturbances of embryogenesis; double-yolk egg; hatchability,

Changes of Pekin Duck Hatchability During the Year

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Hatchability results in poultry depend on a wide range of genetic, biological (e.g., flock age), and environmental factors. Despite the automation of microclimate control in poultry reproductive flock buildings and hatcheries, the weather seems to affect hatchability results. This phenomenon has not been analyzed in detail so far, especially in the case of domestic duck artificial incubation.

The analysis included all 871 batches of hatching eggs (252-24,696 eggs/batch) incubated at the commercial hatchery in Wieszowa (E.G.G. Ltd) from 2019 to 2023. Eggs were collected from 10 parental flocks of ducks (Cherry Valley) that began to lay cyclically, so that eggs from four parental flocks of different ages were usually incubated simultaneously. The effect of the month of egg collection on indicators such as fertilization, embryo mortality between days 1 and 7 of incubation (E1-E7), E8-E24, and E25-E28, and hatchability from set and fertilized eggs was tested using the Kruskal-Wallis test, followed by a post hoc Dunn test.

All analyzed indicators depended on the month of egg collection ($P<0.05$). The highest fertilization of duck eggs (mean ± SD) was found in November (95.9±1.66%), while the lowest was in spring, in April (93.7±3.64%; $P=0.025$) and May (94.1±3.15%; $P=0.070$), and in late summer, specifically in August (94.0±2.91%; $P=0.002$) and September (94.1±3.53%; $P=0.010$). There was a tendency for early embryo mortality to decrease in summer (June-July), but simultaneously, mortality increased during the hatching period. Embryo mortality in E25-E28 was 13.8±7.20%, 14.6±7.68%, and 15.2±4.99% ($P<0.05$) in June, July, and August, respectively, while it was lowest in April (8.2±2.58%), November (9.7±3.24%), and February (10.0±4.43%). As a result, the highest hatchability from total set eggs was in late winter and spring (February 89.2±5.48%, March 87.8±7.37%, April 91.1±2.82%) and November (89.3±3.32%), while the lowest was in the summer months (June 85.1±8.12%, July 84.0±8.69%, and August 83.5±5.57%).

In summary, the hatchability results of domestic ducks in hatcheries depend on the month of egg collection. The highest hatchability is observed from February to April, while it decreases in the summer months (June to August). This is most likely due to the fact that high temperature and water vapor content in the air make it difficult to maintain proper microclimatic parameters in the setter and hatcher.

Funding: project POIR.01.01.01-00-1010/17

Keywords: Pekin duck, incubation, egg fertilization, embryo mortality

Pullet Hatchability and Quality in Hy-Line Brown Laying Lines: a Data Analysis

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Commercial hatchery data can provide critical information about breeder reproductive performance, the efficacy of management practices, and areas of improvement. In layer breeds, egg storage **(ES)** is more common than in broiler lines. Consequently, the short periods of incubation during ES **(SPIDES)** have been widely adopted, but its efficacy in layer breeds has not been well documented. We conducted data analyses of three commercial hatchery datasets (A, B, C) to describe the pullet hatchability **(PH)**, quality **(PQ)**, and embryo mortality **(EM)** of Hy-Line Brown breeders through the entire (22-75 weeks) production cycle. Observations in each dataset (A, N= 13,193; B, N= 14,473; and C, N= 15,889) spanned the years 2013-2023 (A), 2019-2024 (B), and 2022-2023 (C). For all hatcheries, ES ≤6d were noted as "Fresh" eggs, while ES ≥7d were denoted as Stored/SPIDES. The ES varied per dataset (A=0-25 d, B=2-21d, C=4-10d). Response surface **(RS)** analyses were used to describe the interactive effects of flock age **(FA)** and ES, with or without SPIDES. The SPIDES procedure varied per FA and ES, but the maximum machine temperature was 95°F; the process took 9 hours with 3 hours of egg cooling. Multiple linear regression was used when no interaction effect (P >0.05) was observed. Random effects included individual flocks, years, farms, and hatcheries as blocks. The PH of Fresh eggs has not changed in 11 years, but ES and SPIDES application has increased considerably. Consistently, better (P <0.05) PH, PQ, Mid EM, and Total EM were observed for "Fresh" eggs than Stored/SPIDES. However, considering the largest mean difference was 0.68% (Total EM, Dataset C), SPIDES application was effective in minimizing the detrimental effects of extended ES. For each dataset, no interactions (P >0.05) between FA and ES were observed in the "Fresh" eggs; however, interactions were observed (P <0.05) in the Stored/SPIDES eggs. The RS model for Stored/SPIDES eggs (B) was PH = 29.21 + 0.68*FA + 0.33*ES – $0.008*FA^2$ – 0.005*FA*ES – $0.008*ES^2$ (R2 = 0.86). Mean PH across years and datasets remained consistently high for "Fresh" eggs (A=41.17, B=44.49, and C=41.87, %) and Stored/SPIDES eggs (A=41.08, B=44.27, and C=42.07, %). In summary, FA and ES affect PH and PQ. The application of SPIDES can mitigate the deleterious effects of ES, but the application should consider FA and ES.

Keywords: SPIDES, hatchability, layer breeder, pullet, incubation

Data Analysis of Leghorn Breeder Pullet Hatchability and Quality

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Information published about pullet hatchability **(PH)** and quality **(PQ)** of leghorn lines is scarce. Egg storage **(ES)** is necessary to fulfill the demands of large-layer flocks. Short periods of incubation during ES **(SPIDES)** have become ubiquitous in hatcheries, but its efficacy in Leghorn breeders has not been described. Therefore, two commercial hatchery datasets were obtained to describe the effects of breeder flock age **(FA)** and ES on PH (dataset A, N= 39,618) and PQ (dataset B, N= 1,049) for eggs with or without SPIDES application. Observations in dataset A spanned 2013-2023 and 2022-2023 in dataset B. In each dataset, ES ≤6d were denoted as "Fresh" and ES ≥7d were identified as Stored/SPIDES. Response surface **(RS)** analyses were used to describe the interactive effects of FA (22-75 wks) and ES (0-25 d, dataset A and 3-24 d, dataset B), with or without SPIDES. Random effects included individual flocks, farms, and years as blocks. Multiple regression was used when no interaction was observed ($P > 0.05$). From dataset A, the 2013-14 percentage of "Fresh" eggs was 50.6 and 50.4%, respectively, and 36.6% in 2015. The "Fresh" percentage of eggs in subsequent years leveled off between 20-29%. Concurrently, the percentage of Stored/SPIDES eggs increased from 4 or 13% (2013 and 2014, respectively) to around 55-64%. The RS fitted ($P < 0.001$) for Fresh eggs was PH = 32.64 + 0.58*FA + 0.26*ES - 0.01* FA^2 - 0.04* ES^2 + 0.12*SPIDES (R^2 =0.80). Eggs receiving SPIDES had higher PH compared with non-SPIDES regardless of year; however, the magnitude of improvement in PH varies depending on Year. From dataset B, differences ($P<0.05$) between "Fresh" and Stored/SPIDES eggs were observed for PQ1 and 2. Application of SPIDES to eggs stored up to 24d resulted in a mean decrease in PQ1 (0.68%) and an increase in PQ2 (0.37%). No difference was observed between "Fresh" and Stored/SPIDES for PQ3 ($P>0.05$). In conclusion, the ES has increased in the past 11 years, with a simultaneous increase in SPIDES utilization helping to minimize the detrimental effects of ES. The strategic application of SPIDES should consider FA to optimize both PH and PQ.

Keywords: Leghorn breeder, pullet quality, incubation, SPIDES, hatchability.

Interpretation of Hatchery Breakout Data

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Breaking out the unhatched eggs after the incubation process is very informative, as well as examining the eggs containing apparently infertile or dead embryos during the incubation process. It can help to identify possible causes for low hatchability and poor-quality chicks. In most hatcheries, breakouts are performed in a more or less standardized way, either as a routine procedure or as a check when the results are not according to expectations.

Although the procedure is very well described and by itself not difficult to perform, the interpretation of the data can sometimes be an unexpected challenge. When the same person does the procedure as a routine, we can expect that the data and conclusions from different observations will be interpreted in the same way and can be compared. But when more people are involved or if we compare results from different hatcheries, we need to ensure that we are comparing apples to apples.

An additional interfering factor is the determination of fertility. Different breakout procedures (candling at 7-10 days, candling and opening clear eggs, opening hatch debris, or using data provided by the transfer machines at 18 days of incubation) will estimate different fertility levels on the same set of eggs. As many key figures are based on fertility, the outcome might vary based on the breakout procedure being used. This makes it challenging to compare data between different operations.

Besides that, the level of fertility can also influence our conclusions, as embryo mortality is often expressed as a percentage of the number of eggs on the trays examined. But as infertile eggs do not contain an embryo, the same number of early dead on a low fertility or high fertility flock should lead to a different interpretation. 5 dead embryos on a tray is more severe if there are only 50 fertile eggs (10% of the embryos died) on that tray instead of 100 fertile eggs (5% of the embryos died).

To be able to turn breakout data into useful information and learn from it, we must know what we are actually measuring. Just comparing the key figures without looking into the background of the data can be misleading.

Keywords: hatchery break out, data analyses, data interpretation

Session 7:

Management and Connecting the Dots

(Chair – Barbara Tzschentke)

Effect of Broiler Breeder Female Stocking Density during the Laying Period on Egg Production, Mortality, and Hatchability

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The purpose of this research was to determine the effect of two different female stocking densities during the laying period (from 26 to 59 wk of age) on egg production, hen mortality and hatchability of broiler breeder hens. Males and females were grown sex-separated in light-controlled facilities with an 8–h photoperiod. The feeding and body weight programs were carried out as described by Aviagen (2023). In this experiment, birds were randomly transferred from rearing houses to 4 closed fan-ventilated commercial broiler breeder laying houses with a female (Ross 308) stocking density of either 5 females/m² **(CSD)** or 6.6 females/m² **(HSD)**, at 22 wk. At the beginning of the production, female feeder space was 11.32 cm/female or 15.13 cm/female for HSD and CSD groups, respectively. In HSD houses, there were 9.5 females and 5.3 females per each nipple and nest hole, respectively. There were 7 females/nipple and 3.7 females/nest hole in CSD houses. All eggs and dead hens were recorded daily from each house to calculate total percentage of mortality and egg production/hen (HW) week. Eggs from both treatments were incubated in a commercial hatchery to determine hatchability of set eggs and the second grade chick percentages of the treatments. The sorting of chicks into first and second grade was done by the hatchery personnel and the first grade chicks were counted by automatic system. The data were analyzed by One-Way ANOVA procedure of SAS and a Z-test was employed to determine existence of differences in two proportional mortality values. Egg production was reduced 1.2 % (HW) in HSD from 26 to 59 weeks of age ($P > 0.05$). In addition, hen mortality for the same period was significantly higher in the HSD treatment than the CSD treatment (6.34% vs 5.21%; $P = 0.001$). Cumulative hatchability was significantly lower in eggs collected from HSD treatment when compared with CSD treatment ($P < 0.05$), but there is no significant difference in the percentage of second grade chicks of the treatments ($P > 0.05$).

These results indicated that a 30% increase in stocking density from 5.0 to 6.6 females/m² during production period reduced the female feeder space and influenced the performance of broiler breeders. Birds at higher density producing fewer eggs (177.5 vs 181.5 eggs) and chicks (148.3 vs 154.1 chicks) per female broiler breeder. However, total egg or chick production per m² were higher in HSD compare to CSD treatment.

Keywords: stocking density, laying period, broiler breeders, egg production, hatchability

Hatchability and First Week Mortality were Severely Impaired After Inoculation of 18-day-incubated Embryonated Broiler Eggs with *Escherichia coli* and *Enterococcus faecalis*

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in ovo vaccinations have been widely used in the poultry industry in the last three decades. During this vaccination process, various factors, including bacterial contamination of embryonated eggs, can negatively impair hatchability, chick quality and first week mortality. The aims of this study were to assess which bacterial strains affects hatchability, chick quality and first week performance. The effects of different bacterial concentrations and the site of inoculation on the same parameters were determined. Three experiments were conducted. In all experiments, 18 days incubated embryonated broiler eggs were inoculated with 0.1 ml suspension or peptone physiological saline, 36 eggs per group were used in experiment 1 and 2 and 114 eggs per group in experiment 3. Hatching rate, time of hatch, chick length, Pasgar score, mortality, time of death and chick weight at the end of the experiment were determined. Two different isolates of *Escherichia coli* and *Enterococcus faecalis*, isolated from *in ovo* vaccinated unhatched eggs, and combinations of both were used in experiment 1. Per egg 106 colony forming units (cfu) of the bacteria were inoculated. The most virulent strain of *E. coli and E. faecalis* were used in experiment 2 and embryonated eggs were inoculated with three different bacterial doses (102, 104 or 106 cfu/egg) of these bacteria. In experiment 3, *E. faecalis* (104 cfu/egg) was inoculated in the amniotic cavity or in the embryo. The observed hatchability in all experiments was very low (0-19%) in the *E. coli* inoculated groups and moderate (48-69%) in the *E. faecalis* inoculated groups. In experiment 2, E. coli was pathogenic in all used doses and no chicks hatched in these groups. Hatchability did not significantly differ between the different doses when *E. faecalis* was inoculated, and ranged from 56 to 69%. Chick quality did not differ from the control groups in all experiments. First week mortality was in general high (>50%) in all experiments regardless of the bacterial species, dose and inoculation route. Strict hygiene measures should be taken in hatcheries to avoid contamination of eggs with low numbers (<102 cfu/egg) of virulent bacteria, such as *E. coli and E. faecalis*.

Keywords: *in-ovo* vaccination, bacterial contamination, hatchability, chick quality, first week mortality

Understanding the Role of Embryo Development in *in ovo* Vaccination

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Correct timing plays a critical role in *in-ovo* vaccination as it affects the injection site inside the egg, which in turn has direct consequences for hatching performance and the effectiveness of the vaccine. To think about timing, it's important to understand that there can be differences between the incubation time and the estimated development of the embryo. These differences can be caused by many factors, including the age of the breeders as one of them. The aim of this study was to analyze the influence of the age of breeders on the differences between chronological and biological age of the embryos, and its effect on site of injection. The data come from field observations during 2023 and 2024 at a commercial hatchery located in Spain, equipped with single-stage machine from a single supplier. Eggs from breeders of 11 different flocks/ages were analysed. All the eggs were incubated following the same incubation program. A total of 275 embryos had estimated embryonic development age and site of injection evaluated using the Global Hatchery Health Program method®. Statistical analysis was performed using logistic regression followed by posthoc analysis, with embryonic development (in hours) and breeder age (in weeks) as covariates. The results demonstrate significant differences in amnion injections depending on embryonic development. More developed embryos (>444 hours) exhibit less than 50% of injections in the amnion. It was also shown that there is a difference between development inferred by incubation time and embryonic development. A Kruskal-Wallis test followed by post-hoc analysis with breeder age (in hours) was performed and the difference of incubation time minus embryo development was analysed. This difference is significative ($p<0.0001$), showing an overestimated development by incubation time, and is more pronounced in breeders under 30 weeks of age (24h±8.4h), and in breeders over 60 weeks of age (18h±4.6h). Breeders between 30 and 47 weeks of age showed the least affected results (9.3h±9h), although there were two clusters with different behaviour within this age group. This study demonstrates that embryo development plays a critical role in the success of the *in-ovo* vaccination process and calls the importance of better understanding the factors that affect the differences between chronological and biological age of the embryos.

Keywords: *in-ovo* vaccination; embryo development; site of injection

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