IFRG
meeting 2022

Incubation and Fertility Research Group
(IFRG/Working Group 6)

education organization research

October 13 - 14

ifrg
Incubation
& Fertility
Research Group

Hilton Garden Inn Leiden
the Netherlands

2022 IFRG Meeting

Leiden, The Netherlands

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- Conny Maatjens - CIQ Consultancy, The Netherlands

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Preface

IFRG Abstract

Dear friends and colleagues,

We are pleased to welcome you to the 2022 IFRG meeting in Leiden, The Netherlands!

As a working group for incubation and fertility of the World Poultry Science Association (IFRG, WG6), we are grateful and honored to have experts worldwide participate in the meeting to share the latest knowledge. This share-learning platform provides an excellent opportunity for sciences and industry to connect and exchange the latest developments on topics related to fertility and incubation.

This year's program includes two well-known keynote speakers. On the first day, Dr. Kirsten Brady will present "The biological and practical implications of egg storage and SPIDES," and Dr. Henry van den Brand, on the second day, will discuss "Research and implications of eggshell temperature, CO_2 and O_2 during incubation: what do we know?" During the two days, we have professionals with a track record of experience and students to take the podium and introduce us to the latest findings and developments. At the end of the meeting, the Nick French prize will be awarded for the best presentation of a young scientist.

This year, Leiden is the European City of Science 2022, and we provide you with the possibility to experience the beautiful historic city of Leiden.

We hope you all enjoy these coming days!

Best regards,

Organizing Committee
- Ampai Nangsuay
- Conny Maatjens

2022 IFRG meeting; Thursday, October 13th

10:30 - 11:30	**Badge collection**
11:30 - 12:30	**Welcome and lunch buffet**
12:30 - 12:40	**Ampai Nangsuay and Conny Maatjens; Introduction to program**
	Session 1: Embryology, egg storage and SPIDES (Chair; Ampai Nangsuay)
12:40 - 13:25	**The biological and practical implications of egg storage and SPIDES** Kristen Brady (USA)
13:25 - 13:45	**Preliminary study on SPIDES effect of freshly laid goose eggs on hatchability rate** Ewa Łukaszewicz (Poland)
13:45 - 14:05	**Physiological status of embryos from broiler breeder hens under different temperatures during storage period** Serdar Özlü (Turkey)
14:05 - 14:25	**Comparison of the embryo development of the Pekin duck and the Mulard duck** Marcin W. Lis (Poland)
14:25 - 14:45	**Coffee/tea break**
	Session 2: Fertility, egg quality, egg handling and egg storage (Chair; Hilke Willemsen)
14:45 - 15:05	**Feeding barley sprout in broiler breeder roosters: effects on egg fertility and hatchability** Mohammad Hossein Shahir (Iran)
15:05 - 15:25	**Dietary energy-to-protein ratio in the breeder diet affects chick quality** Jesse Heijmans (The Netherlands)
15:25 - 15:45	**The effect of lower egg storage temperature and length of storage period on albumen quality, embryonic development, and hatchability of broiler hatching eggs** Okan Elibol (Turkey)
15:45 - 15:50	Poster session; **Effect of eggs pre-incubation on hatchability and physiological response of ducklings** Magdalena Trela (Poland)
15:50 - 16:10	**Effects of flock age, place of oviposition and cleaning treatments of hatching eggs on contamination and hatchability in broiler breeders** Ron Meijerhof (The Netherlands)
16:10 - 16:30	**Taping of cracks in hatching eggs** Ron Meijerhof (The Netherlands)
16:30 - 16:35	**Introduction to City Tour;** Conny Maatjens
16:45 - 19:00	City Tour
19:30	Conference dinner

2022 IFRG meeting; Friday, October 14th

	Session 3: Incubation and chick vitality (Chair; Conny Maatjens)
09:00 - 09:45	**Research and implications of eggshell temperature, CO_2 and O_2 during incubation: what do we know?** Henry van den Brand (the Netherlands)

09:45 - 10:05	**Observations on nest temperature and relative humidity of Gallus gallus domesticus** Lotte Hebbink (the Netherlands)
10:05 - 10:25	**Impacts of early thermal manipulation on gene expressions at hatch in mule duck** Charlotte Andrieux (France)
10:25 - 10:45	**Using different temperature measuring tools and thermal imaging to monitor cooling during incubation of geese eggs under field conditions** Timea Torma (Hungary)
10:45 - 11:05	**Coffee/tea break**
	Session 4: In ovo sexing (Chair; Barbara Tzschentke)
11:05 - 11:25	**Sexual sorting of chicken embryos using the HINTW gene in allantoic fluid** Simão Santos (Belgium)
11:25 - 11:45	**In ovo sexing technologies for the poultry industry: Insights from 1907 until 2021** Matthias Corion (Belgium)
11:45 - 12:05	**Determining the fertility and the sex of chicken hatching eggs using a new volatile organic compound extraction approach** Matthias Corion (Belgium)
12:05 - 12:15	**Introduction to Combined meeting 2023**
12:15 - 13:15	**Lunch Buffet**
	Session 5: Incubation and early life post hatch (Chair; Eddy van Lierde)
13:15 - 13:35	**Effect of lighting regimes in automatic setters and hatchers on ostrich hatching performance** Zanell Brand (South Africa)
13:35 - 13:55	**A field study on Mycoplasma gallisepticum and EDS 76: effects on egg production, hatchability and chick quality** Juan Carlos Lopez (Panama)
13:55 - 14:15	**Optimal climate of early and delayed fed day-old-chicks in a forced draft situation** Carla W. van der Pol (The Netherlands)
14:15 - 14:35	**Course of hatching chicks in the "on-farm system"** Kamil Kustra (Poland)
14:35 - 14:55	**Coffee/tea break**
	Session 6: Incubation, hatching phase and early life post hatch (Chair; Ampai Nangsuay)
14:55 - 15:15	**Incubation temperature and early feeding affect respiratory resilience of broilers** Jan Wijnen (The Netherlands)
15:15 - 15:35	**Effect of the pull time and the preplacement holding time on yolk sac utilization, the crop filling rate, feeding behaviors and first-week broiler performance** Tolga Erkuş (Turkey)
15:35 - 15:55	**Alternative hatching systems with early access to feed and water; what do we see in the field** Conny Maatjens (The Netherlands)
15:55 - 16:25	**Connecting the dots!** Mike Wineland (USA)
15:25 - 16:35	**Nick French Prize / Closing** ■

The biological and practical implications of egg storage and SPIDES

Kristen Brady[1*], Conover Talbot Jr[2], Julie A. Long[1], Nick French[3], Dinah Nicholson[3] and Murray Bakst[1]

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Cool temperature egg storage prior to incubation is a common practice in the broiler industry; however, prolonged egg storage causes increased embryonic mortality and decreased hatchability. Exposing eggs to short periods of incubation during egg storage (SPIDES) reduces the adverse hatchability consequences of prolonged storage in an applied setting, though the basic mechanisms regulating blastodermal cell viability and apoptosis are poorly understood. To define the impact of prolonged storage and SPIDES, transcriptome analysis compared gene expression from blastoderms isolated from eggs exposed to the following treatments: control (CR, stored at 17°C for 4 days), prolonged storage (NSR, stored at 17°C for 21 days), SPIDES (SR, stored at 17°C for 21 days with SPIDES), and incubated control (C2, stored at 17°C for 4 days followed by incubation to HH (Hamburger–Hamilton) stage 2, used as the ideal standard development) (n = 3/group). Data analysis was performed using the CLC Genomics Workbench platform. Functional annotation was performed using DAVID and QIAGEN Ingenuity Pathway Analysis. Differential expression output from the comparison of CR and C2 groups was used to remove normal embryonic development genes from the comparison between NSR and SR groups. A total of 1229 differentially expressed genes (DEGs) were identified between NSR and SR groups ($q<0.05$, FPKM>20, |fold change|>1.5). The NSR group showed enrichment of gene ontology (GO) terms associated with protein catabolism, mitophagy, endoplasmic reticulum stress, and apoptosis. The SR group showed enrichment of GO terms associated with the TCA cycle, chromatin dynamics, DNA repair, and gene/protein expression. Pathway and network analysis revealed linkages between DEGs and cell death and survival, cell cycle, RNA post-transcriptional modifications, cellular assembly and organization, metabolic function, and DNA replication, recombination, and repair processes. Upstream analysis identified 71 potential regulators of downstream gene expression differences exhibiting significant predicted activity and expression changes between NSR and SR groups. Prolonged egg storage (NSR) resulted in enriched cell stress and death pathways; while SPIDES (SR) resulted in enriched basic cell and anti-apoptotic pathways. New insights into DNA repair mechanisms, RNA processing, shifts in metabolism, and chromatin dynamics in relation to egg storage treatment were obtained through this study. Although egg storage protocols have been examined through targeted gene expression approaches, this study provided a global view of the extensive molecular networks affected by prolonged storage and SPIDES and helped to identify potential upstream regulators for future experiments to optimize egg storage parameters.

Keywords: blastoderm, chicken, egg storage, prolonged storage, SPIDES, transcriptomew.

Preliminary study on SPIDES effect of freshly laid goose eggs on hatchability rate

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Our earlier experiments on stages of embryo development at oviposition in the freshly laid goose eggs indicated great varieties in distribution of the developmental stages from stage VI of EGK to stage 2 of HH (Lukaszewicz et al., 2017, 2019). These stages depended on genotype, flock age and period of the reproductive season. Fasenko et al. (1992) described that older broiler breeder hens tended to produce more developmentally advanced embryos, that were also more resistant to storage. Knowledge about the significance of embryo stage at oviposition on resistance to length of storage and further hatchability inspired us to determine if SPIDES (Short Periods of Incubation During Egg Storage) of goose eggs prior to storage will improve their hatchability. Hatching eggs were collected from Bilgoraj goose kept in Wroclaw University of Environmental and Life Science, and at the day of oviposition divided into two groups: - control (not heated, stored for 10 days) and SPIDES (heated four hours at temperature at 37.5°C, then stored for 10 days). All eggs were placed horizontally on trays, stored at 17°C and air humidity 55-60%, and turned once a day along the long axis. After 10 days eggs were set into incubator and incubated according to guidelines for goose egg, candled on day 10 and 27 of incubation and the unfertilized eggs and eggs with dead embryo were removed. Hatching indexes were determined after 30 days of incubation.

Group	Set eggs (pcs)	Fertility (%)	Dead embryos		Hatched goslings (pcs)	Hatchability from set eggs (%)	Hatchability from fertile eggs (%)
			(pcs)	(%)			
Control	65	76.90	21	32.31	30	46.15	76.92
SPIDES	55	81.82	13	23.64	32	58.1	81.82

Results obtained indicate the positive effect of SPIDES on goose egg hatchability.

Keywords: goose, SPIDES, egg storage, hatchability.

Physiological status of embryos from broiler breeder hens under different temperatures during storage period

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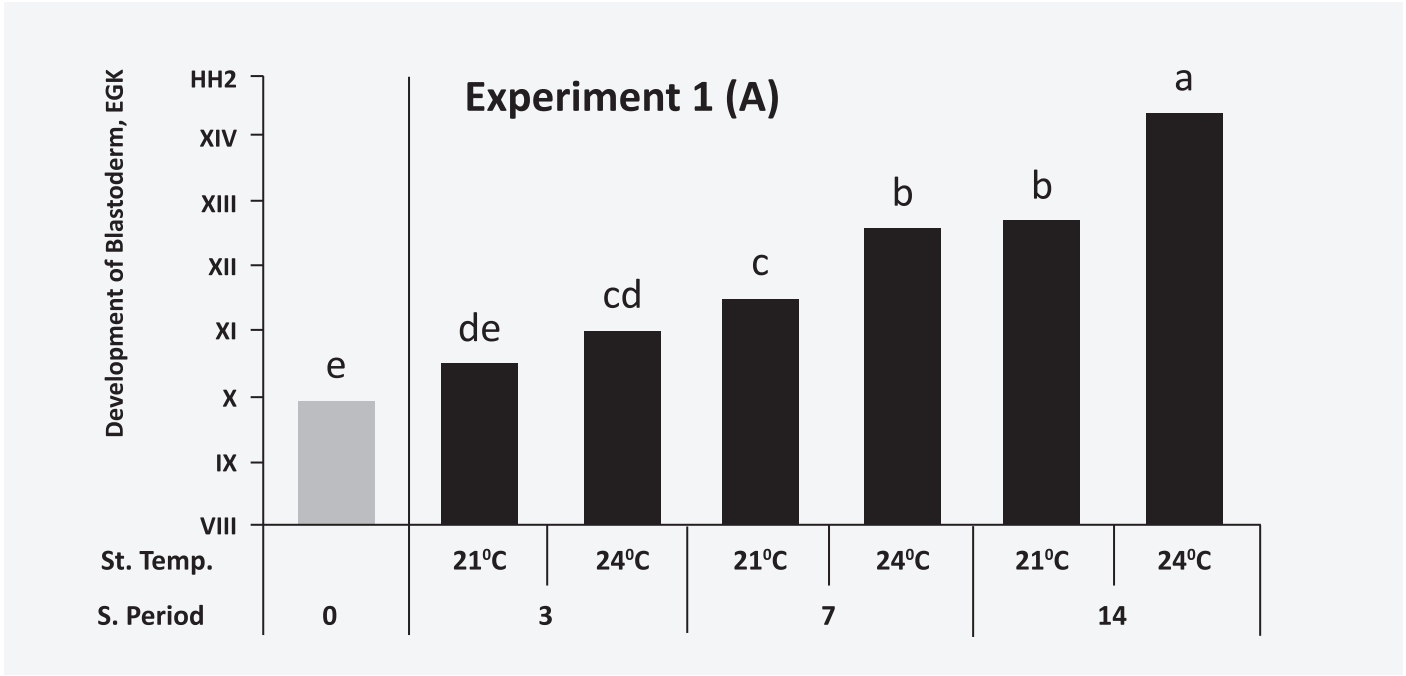
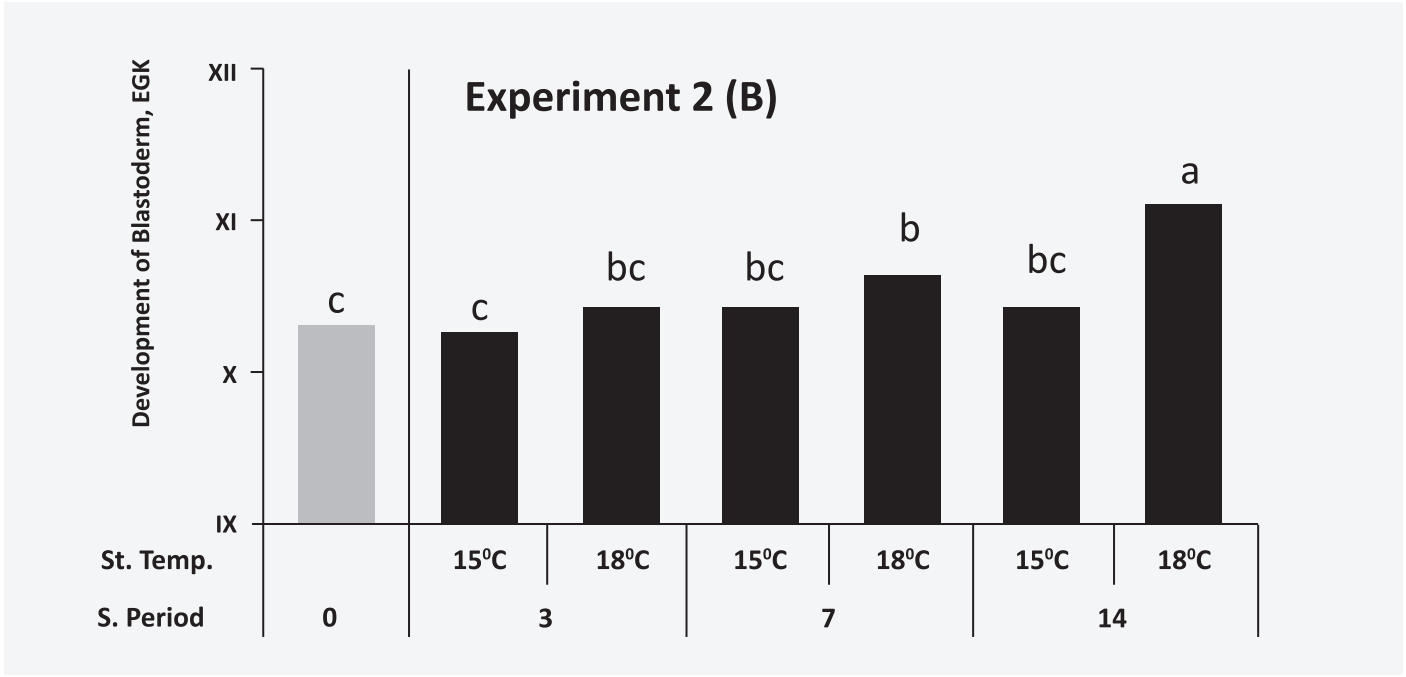
The definition of "physiological zero" first was introduced in 1902 as the temperature below which there is no embryonic development. More recently, "embryonic diapause" term revealed as an alternative for the traditional "physiological zero" that some cellular metabolic processes continue, but gross morphological changes are arrested. The study was conducted to better understand the basis of these changes in embryos during storage period in different hen ages. Hatching eggs were obtained from Ross 308 broiler breeders at 31 wk (**young**) and 46 wk (**prime**) of age in Exp 1, and at 32 wk (**young**), 42 wk (**prime**), and 60 wk (**old**) of age in Exp 2, respectively. A total of 280 eggs that had been laid within a 15 min period were collected from each flock and randomly placed to plastic egg trays in both experiments and then transferred by vehicle with air condition to an experimental egg storage facility (Applied Poultry Research Center at Ankara University, Türkiye) within 2 h. The eggs were randomly assigned to two identical chambers were stored **LOW** or **HIGH** temperature treatments for 14 d and 67-69% RH. The **LOW** and **HIGH** treatment cabinets were set 21 and 24°C in Exp 1, respectively. In Exp 2, 15°C was set for the **LOW** treatment and 18°C was set for the **HIGH** treatment. To assess blastoderm development a randomly subset of 40 eggs were opened before and during storage period (3, 7, and 14 d) (Totally 560 and 840 eggs in Exp 1, and 2 respectively). The data were subjected to one-way-ANOVA using the Minitab 14.0. The significant differences among the flock age × the storage temperature × the storage duration combination means were determined by Duncan's multiple range test at 0.05 significance level. The average blastoderm developments of the duration treatments were significantly advanced by increasing storage period in both temperature treatments in Exp 1 ($P < 0.05$). As expected, the highest average advanced embryo was observed from the eggs which stored 14 d under 24°C temperature, but there was no significant difference between **LOW** (21°C) and **HIGH** (24°C) temperature treatments at 3 d stored eggs (**Figure 1A**). In Exp 2, the blastoderm development average was significantly higher in eggs which stored 14 d under 18°C than 3 and 7 d stored eggs ($P < 0.05$), but there was no significant difference among groups under 15°C conditions ($P > 0.05$). Moreover, there was no significant difference between 15 and 18°C storage temperature conditions at 3 and 7 d of storage period for blastoderm development, but the blastoderm development was directly affected as a result of a 3°C increase temperature for 14 d storage duration (**Figure 1B**). It was also found that the blastoderm development in the **LOW** and **HIGH** storage temperature treatments had a similar trend in the improving in eggs from all flock ages in this study.

In conclusion, the data from this study demonstrated that storage temperatures (18, 21 or 24°C) after lay advanced the stage of blastoderm development in eggs from broiler breeder flocks, but the blastoderm development was not changed at 15°C up to 14 d storage. The current study characterized the critical temperature for chicken embryo as the start of the diapauses period, when the eggs were held at 15°C storage conditions.

Keywords: physiological zero, storage temperature, blastoderm development, flock age.





EGK: Eyal-Giladi and Kochav (1976)

a-e: Groups with different letters are significantly different in each experiment ($P < 0.05$).

Figure 1. The development of blastoderm in Exp 1 (A) and Exp 2 (B).

Comparison of the embryo development of the Pekin duck and the Mulard duck

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Knowledge of the stages of avian embryo development is essential for the supervision of the incubation process in a hatchery. Therefore, it seems interesting and necessary for poultry practice and science to describe the embryo development of the Pekin and Mulard ducks and indicate the stages with an increased probability of embryo mortality during artificial incubation.

The hatching eggs of Pekin (Cherry Valley) and Mulard ducks (1800 eggs/breed) were incubated (setter/hatcher IGLOTECH, Poland) at a gradually decreased temperature (from 37.8 to 36.2°C) and RH 65-60%. Embryological sampling (3 eggs/breed/collection) was on: 0-4, 5-12 and 3- 28/32 days of incubation (d.i) every 6, 12 h and 24 h, respectively. The incubation of the remaining eggs (2 × 1600 eggs) continued until the ducklings hatched. All discarded during candling and unhatched eggs were breakout analysed.

The faster embryo development of Pekin duck in comparison to Mulard was already detected on 1 d.i. Two clear peaks of embryo mortality were observed for both breeds of ducks. However, the 1st peak occurred for Pekin duck between 2 and 6 d.i. (with a critical period in 2-3 d.i., HH 11-17) and was very sharp (74.3% of all deaths), while for Mulard between 2 and 7 d.i. (with a critical period in 4d.i., HH 16-17) and was milder (28.6% of all deaths). The 2nd peak was found for Pekin duck (17.7% of all deaths) between 22 and 26 d.i. (with a critical period during the internal and external pipping), while for Mulard (57.3% of all deaths) between 24 and 30 d.i. with the sharp mortality during the external pipping (29 d.i.).

From the beginning of incubation, the subsequent stages of embryonic development of the Mulard are observed later than in the Peking duck. Moreover, both breeds of ducks are characterized by the different patterns of the timeline of embryo mortality. For this reason, the incubation program needs to be targeted individually for each with these breeds.

Keywords: water fowl, embryogenesis, embryo mortality, incubation

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Feeding barley sprout in broiler breeder roosters: effects on egg fertility and hatchability

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Egg fertility and hatchability are the main concerns of broiler breeder producers. To maintain the fertility and hatchability of the eggs of a breeder flock, rooster's nutrition is of special importance. As the age of rooster increases, usually from the 45 weeks onwards, the percentage of fertile egg and hatchability decreases. Various strategies have been proposed to improve the reproductive performance of aged broiler breeder roosters; one of these strategies is the use of barley sprouts in feeding breeding roosters, which has been used in some farms in Iran. Feeding barley sprout seems to improve fertility in roosters but no research has been conducted on the subject.

This experiment was conducted in the form of a completely randomized design, with two treatments and 7 replications (with 5 roosters and 62 chickens in each replication). Experimental treatments were a control group in which roosters were fed basal ration without feeding barley sprout, and the experimental group in which roosters were fed basal ration plus daily ten grams of barley sprouts. The experiment was conducted from 45 until 53 weeks of age. At the middle (49 weeks) and end of the experiment (53 weeks), one thousand eggs from each group were collected and sent to a nearby commercial hatchery. The fertility of the hatching eggs was determined at the transfer by candling and at the end of hatching process by troubleshooting. After hatch, first and second grade chicks of each experimental group were counted and hatchability percentage were calculated. Troubleshooting was performed on unhatched eggs for the calculation of hatchability in fertile eggs.

The results showed that fertility improved (92 ± 0.90 *vs.* 95 ± 0.60, $P < 0.005$) by feeding barley sprout at the middle phase. Total hatchability increased by supplementation of barley sprout (79 ± 1.3 *vs.* 84 ± 1.1, $P < 0.05$). Hatch of fertile eggs was higher at the end of study in the experimental group (88±1.1 *vs.* 92±0.8, $P < 0.003$). First grade chick percentage increased, at both period, by feeding barley sprout (77 ± 1.3 *vs.* 81 ± 1.1 and 74 ± 1.4 *vs.* 81 ± 1.2, respectively; $P < 0.05$). In conclusion the results of the present study indicating that barley sprout can be used as an inexpensive ingredient for improving the performance of broiler breeder roosters.

Keywords: barley sprout, male, broiler breeder, performance.

Dietary Energy-to-Protein Ratio in the Breeder Diet affects Chick Quality

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This study aims to investigate the impact of breeder dietary energy-to-protein ratio on fertility, hatchability, embryonic mortality and chick quality. At d 0, 1,200 broiler breeder pullets (Ross 308) were randomly assigned to 4 treatments in 20 pens in a 2 x 2 experimental design. The pullets were subjected to two rearing diets (0-21 wk of age) and two production diets (21-60 wk of age) with a different energy content (96% and 104% AME_n relative to breeder recommendations), which were fed on a pair-gain principle. At 32, 36, 40, 45, 50 and 55 wk of age, 150 settable hatching eggs per age were selected per pen for incubation at a commercial hatchery. At E18 and at pull, clear eggs and unhatched eggs were opened to determine infertility or stage of embryonic mortality. At pull, chicks were classified as first or second grade and a subsample of chicks (n = 24 to 46 chicks per pen per age) were weighed. Data were analysed by linear mixed models including rearing and production diet and their interaction and breeder age in the model. Not normal distributed data were arc sin transformed to obtain normal data. No interactions were observed. Breeder rearing and production diet did not affect fertility, hatchability, embryonic mortality or chick weight. Feeding a 104% AME_n production diet reduced the percentage of second grade chicks, compared to feeding a 96% AME_n production diet (2.2 vs. 3.5%, respectively, P = 0.003). Breeder age affected reproductive parameters, where older breeders (> 40 wk of age) had a 12.8% lower fertility, 2.0% lower hatchability of fertile eggs, 2.0% higher embryonic mortality, 2.5% higher percentage of second grade chicks, and 7.2 g heavier day-old chicks compared to younger breeders (≤ 40 wk of age; P < 0.001). It can be concluded that a higher energy diet of breeder hens during production has beneficial effects on chick quality.

Keywords: Second grade chicks, breeder nutrition, fertility, hatchability.

The effect of lower egg storage temperature and length of storage period on albumen quality, embryonic development, and hatchability of broiler hatching eggs

Okan Elibol[1*], Serdar Özlü[1], Rana Dişa[2], Tolga Erkuş[3], and Dinah Nicholson[3]

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This study investigated the effect of lower storage temperature on the albumen quality, the blastoderm development, and the hatchability of short and long stored broiler hatching eggs. In this study, freshly collected hatching eggs from a commercial flock of Ross 308 at 57 wk of age were stored for 2 d at 0°C, 4°C, 8°C, and 16°C (control) in four identical temperature controlled rooms. After the first 2 d, the eggs from all groups were also stored for 2 d or 12 d at 16°C before incubation. Therefore, total egg storage periods were either 4 d or 14 d. The albumen quality (height and pH) and developmental stages of the blastoderm before and after the first 2 d of the storage were determined. There were 8 sub-treatment groups comprising 4 storage temperatures (ST) groups × 2 storage periods (SP). There were 10 replicates (150 eggs per tray) per ST × SP subtreatments, with a total of 12,000 eggs were set in a single stage incubator in a commercial hatchery. All unhatched eggs were opened and examined macroscopically to determine fertility or embryonic mortality (early dead [0 to 7 d], middle [8 to 17 d], late dead [18 to 21 d plus pipped eggs]) to calculate the percentage hatchability of fertile eggs.

In this study, as expected, the eggs stored for 14 d had a significantly lower hatchability of fertile eggs owing to increase the percentage of early embryonic mortality, contaminated eggs and the second grade chicks than the short-period stored (4 d) eggs ($P<0.05$). There was a significant difference in the albumen height and pH for the storage temperature groups ($P < 0.01$). Albumen height was higher at 0°C compare to fresh, 4°C, 8°C and 16°C (control) groups whereas the albumen pH increased at 16°C compare to the other storage temperature groups at 2 d of the storage period. There was no significant difference in the embryonic development due to the storage temperature. For the fertile hatchability, a significant interaction was noted between ST and SP, which showed that the ST had no effect in 4 d stored eggs, whereas 0°C group had a significantly lower fertile hatchability compare to the other ST (4°C, 8°C and 16°C) groups when the storage period was extended (14 d).

It can be concluded that lower storage temperature (between 8°C and 0°C) improved the albumen quality compared to control temperature (16°C). Furthermore, hatchability was affected negatively only stored at 0°C during the first 2 d of 14 d storage in eggs from the older flock.

Keywords: storage temperature, storage period, albumen quality, hatchability.

Effects of flock age, place of oviposition and cleaning treatments of hatching eggs on contamination and hatchability in broiler breeders

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Floor eggs are of economical importance within the broiler breeder industry since because of the higher risk of contamination and lower hatchability. The aim of this study was to establish the effects of the flock age and place of oviposition on hatching parameters, and to investigate whether cleaning practices can improve the incubation results of floor eggs. A total of 4950 eggs were collected from two commercial Cobb 500 breeder flocks at the age of 29 and 64 weeks. Eggs were divided into groups depending on the place of oviposition (nest eggs, floor eggs or eggs laid in the nest and put on the floor after cooling). Floor eggs were further divided on eggs with visually clean or dirty shells. Dirty floor eggs were cleaned either by washing or brushing the eggshell. After incubation the basic hatching parameters were measured (fertility, egg weight loss, embryo mortality, contaminated eggs, hatching percentage) as well as the day-old chick weight and length. The results showed that hatchability rate deteriorates with advancing bird age and that place of egg cooling is a critical point for contamination. Eggs that are cooling down in the litter have a higher risk of contamination and reduced hatchability than eggs that are cooling down in the nest, even if they are afterwards placed in the litter. The results also indicate that floor eggs that do not show visible contamination still have a significantly higher degree of contamination and lower hatchability compared to nest eggs. The effect of cleaning procedures like brushing or washing is limited, although in this experiment brushing of the shell of floor eggs reduced the level of contamination and early embryonic mortality in the young flock, although this reduction did not significantly affect the overall hatchability.

Keywords: flock age, place of oviposition, cleaning treatments, contamination, incubation.

Taping of cracks in hatching eggs

Ron Meijerhof[1*], Lidija Peri[2], Mirjana Mitrakovic[3], Biljana Tomic[4]

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In broiler breeder production, up to 2% of hatching eggs are rejected because of cracked or broken shells. Eggs with cracks give a reduced hatchability and a lower chick quality and cause economic loss. The main goal of this study was to determine the effect of sealing eggshell cracks with surgical tape on hatching parameters. A total of 3,000 eggs (Cobb 500, 34 weeks old) was used in the experiment. All eggs were stored for 4 days prior to setting. 600 intact eggs represented control. The other eggs were artificialy cracked by the operator either on the first (1200 eggs) or on the last day of storage (1200 eggs). In both groups, cracks on 600 eggs were sealed by adhesive surgical tape while the other 600 cracked eggs remained untreated. Within each experimental group, eggs were assigned randomly to 4 setter trays representing 4 replicates of 150 eggs. The egg weight loss during incubation was the highest ($P < 0.01$) in groups of non-sealed cracked eggs. The egg weight loss in the cracked and sealed groups was higher compared to the control group ($P<0.01$), but was within normal limits. Percentage of egg contamination was not different between groups. Embryonic mortality was higher in all stages of embryonic development in eggs that were cracked and not sealed ($P<0.01$) compared to the cracked and sealed eggs and the control group. Hatching percentage was significantly lower in non-sealed groups ($P<0.01$) compared to sealed groups and positive control. No siginificant difference in hatching parameters was observed between sealed groups and positive control, regardless of moment of cracking, indicating that surgical tape can be used for sealing cracks on the eggshell to support embryonic survival, both on cracks that occur at the farm and during handling in the hatchery.

Keywords: incubation, cracked egg, sealing, surgical tape.

Research and implications of eggshell temperature, CO_2 and O_2 during incubation: what do we know?

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In artificial incubation, hatchability and hatchling quality can be influenced by animal related factors, such as breeder age and by environmental factors, such as temperature and gas concentrations. In this presentation, focus will be put on eggshell temperature and on CO_2 and O_2 concentrations, during several phases of incubation. To optimise eggshell temperature, the incubator temperature needs to adjusted continuously to cope with changes in metabolic heat production by the embryo and by evaporative heat loss through the eggshell. Metabolic heat production is in turn related to oxygen availability, which is related to the eggshell conductance. Suboptimal incubator temperature in early incubation has been associated with retarded embryonic development (particularly at too low temperatures) and teratological effects, expressed in a higher percentage of malformed embryos (particularly at too high temperatures). Suboptimal incubation temperate in late incubation has been associated with delayed hatching and lower robustness in later life (low temperature) and higher embryonic mortality, poor hatchling quality and higher risk on metabolic disorders in later life (high temperature). Based on numerous studies the current advice for optimal eggshell temperature is 37.5 to 38.0°C throughout incubation. CO_2 concentrations has been adjusted during different stages of incubation. Concentrations up to 4% or even higher have been used, but quite often adjustments in CO_2 concentrations are confounded with changes in relative humidity, temperature and O_2 concentration. Recently, we have demonstrated that effects of CO_2 concentration up to 0.8% after day 8 of incubation had limited effects on embryonic development and hatchling quality appear as long as the other factors were maintained constant. Higher CO_2 concentrations during late incubation might force hatching, with risks of premature hatching and poorer hatchling quality. Oxygen concentration is particularly of interest in relation to incubation on high altitude. With lower oxygen pressure, O_2 availability for the embryo is reduced. To maintain the balance between embryonic metabolism and oxygen availability, it has been suggested to reduce the eggshell temperature, because otherwise the risk of overheating in late incubation is considerable. It can be concluded that incubation is a sensitive combination of temperature, CO_2 and O_2 and the hatchery manager can strongly influence the hatchability and hatchling quality by playing with these factors.

Keywords: carbon dioxide, eggshell temperature, hatchling quality, incubation, oxygen.

Observations on nest temperature and relative humidity of Gallus gallus domesticus

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We draw inspiration from nature for many of our innovations. Although there are some studies available on avian nest temperatures and incubation behaviour, there is a lack of data on natural incubation behaviour and nest temperatures (NT) of *Gallus gallus domesticus.* Therefore, the objective of this research was to study several chicken nests to obtain more data.
In the spring of 2021 and 2022 a total of 19 different chicken nests of different breeds, housing conditions and social flock situations were equipped with egg-shaped Madgetech dataloggers which measured air temperature and relative humidity. Two nests were also equipped with cameras.
The average NT was 33.3°C with a standard deviation (SD) of 7.72. Average relative humidity was 43.4% with a SD of 10.9. Average NT fluctuated during the day, roughly following a sinusoidal wave with lowest temperature at 9 a.m. and highest at 9 p.m. NT was highest on the first and last incubation day but it fluctuated throughout incubation. There was a low positive correlation between hatching success and NT and a low negative correlation between hatching success and SD. Regularly, NT drops 2 times SD below average. It was validated by cameras that the hen does not leave her nest during those periods but that the sensor was not completely covered by the hen. As the hen turns her eggs regularly, the sensor was usually put back in the centre of the nest during her next turning activity.
It would be interesting to study whether periods of cooler incubation temperature or deliberate fluctuation can be beneficial for commercial incubation results. However, the nest temperatures from this study cannot be directly translated for use in incubators as in natural incubation the heat transfer is mainly via conduction instead of convection in incubators. Fluctuations as large as measured in this study will lead to unacceptable hatch losses in commercial incubators, but maybe small and controlled fluctuations on daily basis is interesting to investigate.

Keywords: natural incubation, nest, temperature, relative humidity, *Gallus gallus domesticus.*

Impacts of Early Thermal Manipulation on Gene Expressions at Hatch in Mule Duck

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Temperature changes during the embryogenesis are well known to modify either thermotolerance of broilers or hepatic metabolism in mule ducks. This study will focus on the impact of the temperature increase (+1°C, 16h/24, from embryonic day 13th to 27th) in mule ducks. We firstly analysed the direct impact of temperature changes during the embryogenesis. One hour after the increase of temperature, relative expressions of genes involved in lipid synthesis, thyroid and inflammation pathways were up-regulated. Heat shock proteins (HSPs) were also significantly stimulated by the thermal manipulation, with up and down regulations depending of the studied HSP gene. These genes were also significantly modified after days of temperature increase, confirming a direct regulation of gene expressions by the thermal manipulation. Indeed, lipid, inflammation, cell proliferation, thyroid and heat shock protein genes were up-regulated at the embryonic days 16th, 20th and 24th. Only one gene involved in epigenetic mechanisms was down-regulated at the embryonic day 20th. In order to test if the thermal manipulation could have a permanent effect later in life, we also analysed gene expressions at hatch, this period corresponding to a critical period for the animals. In relation to the early thermal manipulation, animals presented lower weights at hatch but also lower gene expressions for genes linked to heat shock proteins and thyroid pathway. Moreover, genes involved in lipid metabolism and inflammation were up-regulated. Our results strongly suggest a long-term effect of the early thermal manipulation in ducks. Further studies are now in progress to understand the mechanisms of the observed programming in hatching ducks.

Keywords: Mule duck, thermal manipulation, gene expression, heat shock protein, metabolic programming.

Using different temperature measuring tools and thermal imaging to monitor cooling during incubation of geese eggs under field conditions

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Monitoring the eggshell temperature in the setter, with different measuring tools at critical points is widely used in chicken incubation, however the literature on the use of the same tools in incubation of goose eggs is limited. One of the most critical points during incubation of goose eggs is cooling and spraying the eggs outside the setters during the second half of incubation, until 84.2-86°F temperature is achieved at the apex.

The aims of this field measurement were to check the applicability of Tinytag loggers, Braun thermometer and Flir thermal camera to monitor eggshell temperature of geese eggs, and to identify the most informative location on the trolley to use as reference point during cooling, preferring the side eggs as they are more accessible. The eggs were measured on the 15th and 22nd day of incubation, at the beginning and at the end of cooling with all devices from 24 individual locations. Egg temperature was measured at the equator of the goose eggs, as common practice in chicken eggs. However, the target temperature of cooling goose eggs during incubation has to be determined at the apex, so with Flir both equator and apex temperature were recorded.
The analysis of the database shows a strong (>0.70) and significant (P<0.05) correlation between:

- Braun measurement at different locations after cooling and apex measurements
- Flir measurements of eggs on different locations on the tray and on the trolley, both measured at the equator and apex of the eggs,
- Side egg apex measurements and temperature measured on the eggs in the middle of the tray at the beginning and the end of cooling at any location on the trolley

Further analysis of the Flir data shows a strong (0.91) and significant (P<0.001) linear correlation between the measurements at the equator and at the apex of the egg. Linear regressions (R^2= 0.8142) were determined using apex and equator measurements as dependent :

- Apex °F = 0.9609x Equator °F + 2.5437 and Equator °F = 0.8474x Apex °F + 15.771

It can be concluded that the side eggs on the setter trolleys are suitable to monitor the cooling process if we measure them with Flir thermal camera either at the equator or apex, or use Braun at the equator and convert the value to the apex target using the equations above. For Tinytag this is only valid to the top tray, but it can be caused by the tape we used to fix the sensor, so a re-measurement is required.

Keywords: goose incubation, temperature, Flir, Braun, Tinytag.

Sexual sorting of chicken embryos using the HINTW gene in allantoic fluid

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The poultry industry is undergoing a revolution to end the male-chick culling, pushing for better solutions to meet its requirements: work with all eggs' colors, > 98.5% accuracy, low disturbance and price, sexing before day 7, and > 20,000 eggs/hour. Currently, the most accurate technique relies on PCR using allantoic fluid (AF) from day 9, despite being essential to apply sexing methods before embryo pain perception (day 7). To date, PCR techniques applied before day 9 have focused only on tissue or blood cells, which is highly invasive and impacts hatchability. In our work, we established a PCR protocol based on a female-specific gene in the W-chromosome, *HINTW*, a highly conserved gene. We incubated 80 ISA brown eggs (37.7 °C, 50% RH) and extracted 100 µL of AF from day 6 to 9. Subsequently, purified genomic DNA was amplified with newly designed *HINTW*-specific primers and *DMRT-1* gene primers as control.

First, the melting temperature confirmed the specificity of amplified sequences from female embryos by comparison with the *HINTW* template (82 °C), which was 1.5 °C higher in male samples. Next, based on the cycle threshold, the *HINTW* sequence seemed to be amplified in 100% of the female and 94% of the male samples, the latter possibly related to cells from the mother hen or sequences with similarities at the Z-chromosome. Notably, the difference between the mean cycle threshold of females (mean ± standard deviation = 25.3 ± 2.78) and males (mean ± standard deviation = 35 ± 1.8) was statistically significant ($p < 0.0001$). A linear fitting of the cycle thresholds revealed a 100% sexing accuracy using AF samples before day 7, whereas the gene is amplified before cycle 31 in females. As such, the newly developed assay allows minimally invasive sorting of chicken embryos as early as day 6. Future work will focus on improving sampling strategy and decreasing assay time below the current 60 minutes.

Keywords: in ovo, male-culling, allantoic fluid, sexual sorting, HINTW.

In ovo sexing technologies for the poultry industry: Insights from 1907 until 2021

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In ovo sexing is an approach for sex-sorting chicken embryos before or during incubation. It aims to stop the conventional culling of day-old male chicks, while improving animal welfare in the laying hen industry. Numerous technologies have been reported, with some being also commercially available. However, further optimization is required to achieve desired accuracy and speed before pain onset in chicks, with minimal hatchability impact, and at an acceptable cost. To facilitate further optimization, we reviewed 45 papers and 100 patents to define the actual status of the techniques and highlight current gaps. Consequently, we defined five major non-optical techniques (DNA analysis, genetic engineering, immunosensing, ion mobility- and mass spectrometry [IMS & MS], volatile organic compound [VOC] analysis), and five optical techniques (spectroscopy, being: Raman and fluorescence-, infrared and Terahertz-, visible-near-infrared [Vis-NIR], nuclear magnetic resonance [NMR], morphometric studies).

Concerning actual technology status, diversity was predominantly found in patents with some approaches still requiring additional scientific evidence (e.g. IMS & MS was not reported in papers). Furthermore, genetic engineering, NMR, and VOC analysis were described in only a few papers. In contrast, balanced reporting in papers and patents was observed for Vis-NIR-, Raman- and fluorescence spectroscopy, DNA analysis, and immunosensing. Especially the latter two were the oldest technologies and will most likely continue to be used as standards for *in ovo* sexing.

When reviewing current gaps, invasiveness was considered an important characteristic. Optical techniques are usually non-invasive, except Raman, whilst non-optical are invasive, except VOC analysis. Optical techniques (including Raman) also enable sex detection between days 0 to 3 but with low accuracy. Immunosensing, DNA analysis, or IMS & MS (commercially used) are minimally invasive with high accuracy but are applicable only after day 9. VOC analysis remains promising for early and non-invasive sex-sorting, although more research is required.

In conclusion, none of the technologies complies yet with all the industry requirements, and more research and consensus between consumers, industry, and governments are necessary.

Keywords: *in ovo* sexing, male chick culling, papers and patents review.

Determining the fertility and the sex of chicken hatching eggs using a new volatile organic compound extraction approach

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Recently, the study on volatile organic compounds (VOCs) emitted from chicken hatching eggs gained increasing interest for non-invasive *in ovo* sexing. Traditionally, a solid-phase microextraction fiber is used to extract VOCs from an egg enclosed in a jar. Although its subsequent analysis on a gas chromatography-mass spectrometry (GC-MS) device reaches a desirable signal, eggs need to be sequentially extracted (e.g. delivering 7 measurements per day). Subsequently, this lengthy process limits the observation numbers and impacts statistical robustness. Therefore, we introduce a new extraction approach whereby a PDMS-coated stir bar (Twister®) is enclosed for two hours together with the egg. Resultantly, multiple eggs can be extracted in parallel. Furthermore, these Twisters® can be stored for three days and robotic sampling automatically analyzes these sorbents on the GC-MS, triplicating the measured eggs per day (e.g. 21 eggs vs. 7) and providing a similar signal relative to the fiber approach.
Two experiments were conducted on Isa Brown® eggs that were incubated under standard conditions (37.7 °C, 50 % RH). For the statistical analysis, the dataset was randomized and cross-validated. Next, partial least squares discriminant analysis models were constructed and optimized using variable reduction methods. In the first experiment, the difference in VOC profile was assessed between 10 to 12 fertilized and 4 to 6 unfertilized eggs on the even incubation days between days 0 to 12. As a result, it was possible to assess egg fertilization throughout incubation with an average accuracy of 90 %. In the second experiment, sex determination was performed on respectively 43, 51, and 27 eggs on days 8, 10, and 12. Although a 60.5 % accuracy was obtained on day 8, it was possible to increase it to 85 % on days 10 and 12.
In conclusion, a new extraction approach for egg VOCs was developed using Twisters® and allowed for analyzing egg fertilization or embryonic sex. Future perspectives aim for reproducing experiments on higher numbers and optimizing data preprocessing and statistical analysis.

Keywords: Volatile organic compound profiling, *in ovo* sexing, egg incubation, egg gases, gas chromatography-mass spectrometry.

Effect of lighting regimes in automatic setters and hatchers on ostrich hatching performance

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The environment in which an embryo develops can have a lasting effect on well-being throughout its life. Traditionally ostrich eggs are incubated in complete darkness and are only exposed to light when the incubator is opened. Exposing embryos to light during incubation benefitted hatchability, chick quality and hatching time in other poultry species. The effect of light exposure during incubation on ostrich eggs was investigated with material collected from the pair-bred ostrich flock on the Oudtshoorn Research farm, South Africa. A total of 4042 eggs were set in electronic setters to investigate the effect of different lighting regimes (no light vs. light for 24 hours a day) cool white LED lighting with a colour temperature of 6500 K (900 lm) in setters and hatchers in a completely randomised design. Of these eggs, 3815 were transferred to hatchers at 35 days of incubation, where they were randomly assigned to a 2 (lighting regime in the setter) x 2 (lighting regime in the hatcher) factorial design.

Lighting regimes in the setter reduced (P<0.01) early embryonic mortality (0.051±0.007 in eggs supplied with light vs. 0.082±0.007 in eggs incubated in darkness) and total embryonic mortality (0.264±0.015 in eggs supplied with light vs. 0.319±0.015 in eggs incubated in darkness). When only eggs transferred to the hatcher were considered, a similar tendency was observed for late embryonic mortality (0.202±0.013 in eggs supplied with light vs. 0.223±0.013 in eggs incubated in darkness; P=0.09). In these eggs, embryonic mortality was unaffected by lighting regime in the hatcher (P>0.20) as well as the interaction between incubation regime in the hatcher and the setter (P>0.20). Evaporative water loss to 21 and 35 days of incubation was unaffected by the lighting regime (P>0.05). In contrast to the results for water loss, eggs subjected to continuous lighting during incubation hatched earlier that those incubated in complete darkness (respectively 42.51±0.04 vs. 42.73±0.04 days; P<0.01). Expressed relative to a 24-hour day, these results imply that eggs subjected to lighting hatched, on average, 5.3 hours earlier than those incubated in darkness.

These results suggested that provision of continuous lighting during incubation benefitted embryonic survival in ostrich chicks. The biology underlying this result is not explicitly known, but further studies where alternative lighting regimes are considered may lead to a better understanding of the mechanisms that may be involved.

Keywords: chick weight, moisture loss, pipping time.

A field study on Mycoplasma gallisepticum and EDS 76: effects on egg production, hatchability and chick quality

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Usually when there are problems in hatchability and/or chick quality in the hatcheries we seek the reason in improper managements and no ideal environmental conditions. There are some pathogens that can generate many problems in the normal development of the embryo. *Mycoplasma gallisepticum* and the Atadenovirus causing EDS 76 are some of them. The following field cases describes the effects observed in some hatcheries. Despite strict biosecurity, the flock of turkey hens (around 8000) became infected around week 22 with *Mycoplasma gallisepticum*. The hens did not present any respiratory symptoms. Due to market circumstances, it was decided to incubate the eggs of that flock. Egg production on the farm was normal and contrary to expectations, embryonic mortality did not increase except at the end of the production cycle. It was assumed that vertical transmission would be very low and therefore farm mortality would not be so dramatic, but horizontal transmission increased the problem. Mortality at the third week in the farm reached around 7% in some cases. The confiscation of the birds at week 17 upon arrival at the slaughterhouse was very high up to 30%. In the case of EDS 76 different breeds were affected, with broiler breeders and brown egg birds being more susceptible. Affected hens generally did not show clinical signs. There was a drop in egg laying between 10 to 30%, lasted up to 5 weeks. Wrinkled or misshapen eggs were not observed, only thinner shells, lack of color and some roughness. Initially shelled eggs were not reported in some farms for the workers maybe because the hens ate them. At necropsy, no inflammatory process in the reproductive system was visually observed, as some authors have described. In some hatcheries it was reported that if the eggs with shell alterations were removed the other eggs hatch normally. No alterations were reported in the internal quality of the eggs. Sometimes the birds hatched earlier than scheduled, perhaps due to unnecessary waiting time inside the hatchers, which affected the quality of the birds when they arrived at the farm.

Keywords: pathogen; embryo mortality; misshape eggs.

Optimal climate of early and delayed fed day-old-chicks in a forced draft situation

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Little data is available on the optimal climate of day-old-chicks in a forced draft situation, such as during transportation, and how this is affected by delayed feeding (DF) or direct access to feed and water after hatch (early feeding; EF). Objective was to test various temperature, relative humidity (RH), and air velocity combinations in EF and DF chicks and monitor their behaviors and body temperature. One air temperature (33, 34, 35, 36, 37 or 38°C) was tested with one RH setting (40, 60, or 80%) at 2 air velocities (0.5 or 1.0 m/s) in 36 rounds. Per round, 360 newly hatched Ross 308 from HatchCare (EF) or a traditional hatcher (DF) were divided over 4 baskets/treatment (45 chicks/basket; N = 12,960 chicks), bulk weighed, and placed in a climate controlled wind tunnel. Acclimatization took 30 minutes and then huddling (floor cover), panting, and lying down were observed every 10 minutes for 40 minutes. Afterwards, body temperature was measured in 12 chicks/treatment and chicks were again bulk weighed. Optimal climate was defined as <1% panting, body temperature 40.0-40.7°C, and >80% floor cover. Latent (evaporative) and sensible (contact) heat loss were calculated from sensor and weight loss data. The optimal climate depended on feeding status and RH. EF chicks preferred 35 – 37°C air temperature at 40% RH but only 35°C at 0.5 m/s and 80% RH. DF chicks preferred 35° (at 0.5 m/s) to 36°C (at 1.0 m/s) and this was almost completely independent of RH. Although heat production was almost double in EF chicks, they lost half of their body heat through evaporation, showing that they have a wider ability to actively thermoregulate than DF chicks, which had almost no latent heat loss. This experiment shows that tight environmental control is crucial for all newly hatched chicks and RH should be considered when storing and transporting EF chicks.

Keywords: Thermoregulation, Early feeding, Transportation.

Course of hatching chicks in the “on-farm system”

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“On-farm hatching” is one of the proposed alternatives to traditional hatching.This solution reduces distress and improves the welfare of the chicks around the hatching period. Therefore, it seemed interesting to compare the course of chicks hatching in the traditional system and the “on-farm system”.

Hatching eggs of 50 weeks old parental flock of chicken broiler Ross 308 (Aviagen) were incubated in setter (PasReform) of commercial hatchery (DanHatch Poland JSC.). The eggs were candled at 441 hour of incubation (h.i), and 2250 embryonated eggs were selected nad randomly divided into three parallel groups. The incubation of the control group (5 baskets) was continued in the hatcher. The other eggs were transported (1 h)
into the experimental chicken house (ZD Gorzyń, Poznan University of Life Science), and set on litter (litter group) or plastic trays (tray group) per 38 eggs per pen. Microclimatic parameters during “on-farm” hatching were: temperature 33.5-35°C and RH 18-22%. The course of the hatching was monitored every 3 h since 465 h.i. All unhatched eggs were breakout analysed.
The hatchability in the hatcher was 94.4% in compare to 93.9 and 93.0% for “on-farm” litter and tray groups, respectively (P> 0.05). On the other side, there were found 2.1% crippled or dead chicks in the traditional system, but only 0.1-0.3% “on-farm” system. The chicks started leaving the shell at 470.3±1.48 h.i. in the hatcher while 12.1 and 14.6 hours later in the “on-farm system” in groups of litter and tray, respectively (P <0.05). However, the “hatch window” estimated by linear regression was
(b ± Sb) 28.3± 2.11, 28.0 ± 1.49 and 26.9 ± 1.09 hours (P > 0.05), for the hatcher, litter and tray group, respectively.

In summary, the hatchability of the “on-farm system” is similar to traditional, while the crippling level of the chicks is much lower. The chicks in the “on-farm system” hatch later than the hatcher, but the hatch window is similar.

Keywords: chicken broiler, hatchability, welfare, incubation.

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Incubation temperature and early feeding affect respiratory resilience of broilers

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Avian pathogenic E. coli bacteria can infect the respiratory tract of broilers and cause colibacillosis. This common disease negatively affects welfare and economic profits. Curative antibiotic treatment raises concerns on antimicrobial resistance and therefore additional measures that support broiler resilience against colibacillosis are desired. Access to feed and water directly after hatch (early feeding) may enhance resilience at later ages. Additionally, a high eggshell temperature (EST) during mid incubation may accelerate embryo development and improve chick quality at hatch, supporting potential positive effects of early feeding. To test these hypotheses, eggs from a young Ross308 breeder flock were incubated at constant 37.8°C EST (control) during 21 days or at 38.9°C EST during embryo days 7 – 14 (high) and chicks were subjected either to early feeding or they were fed 48 hours after hatch (delayed feeding) in a 2 x 2 factorial arrangement. Broilers (N = 1,800) were divided over 36 pens and grown for 6 weeks. At day 8 post hatch, colibacillosis was induced by intratracheal inoculation of E. coli. Incidence and severity of local and systemic infection were assessed at 6 moments between 3 hours and 7 days post inoculation by scoring lesions in thoracic air sacs, liver, and pericardium, and by determination of live E. coli in air sacs and blood. Broilers were weighed daily during 13 days post inoculation and weekly thereafter. At high EST, early feeding resulted in higher incidence of systemic infection compared to delayed feeding whereas at control EST, systemic infection did not differ between feeding strategies. Regardless of EST, early compared to delayed feeding resulted in lower incidence of local infection, fewer BW deviations, and higher growth until day 35. In conclusion, early feeding can support certain aspects of respiratory health during an infection and thereby enhance broiler disease resilience, but only when EST during mid incubation is not too high.

Keywords: incubation, eggshell temperature, early feeding, delayed feeding, colibacillos.

Effect of the pull time and the preplacement holding time on yolk sac utilization, the crop filling rate, feeding behaviors and first-week broiler performance

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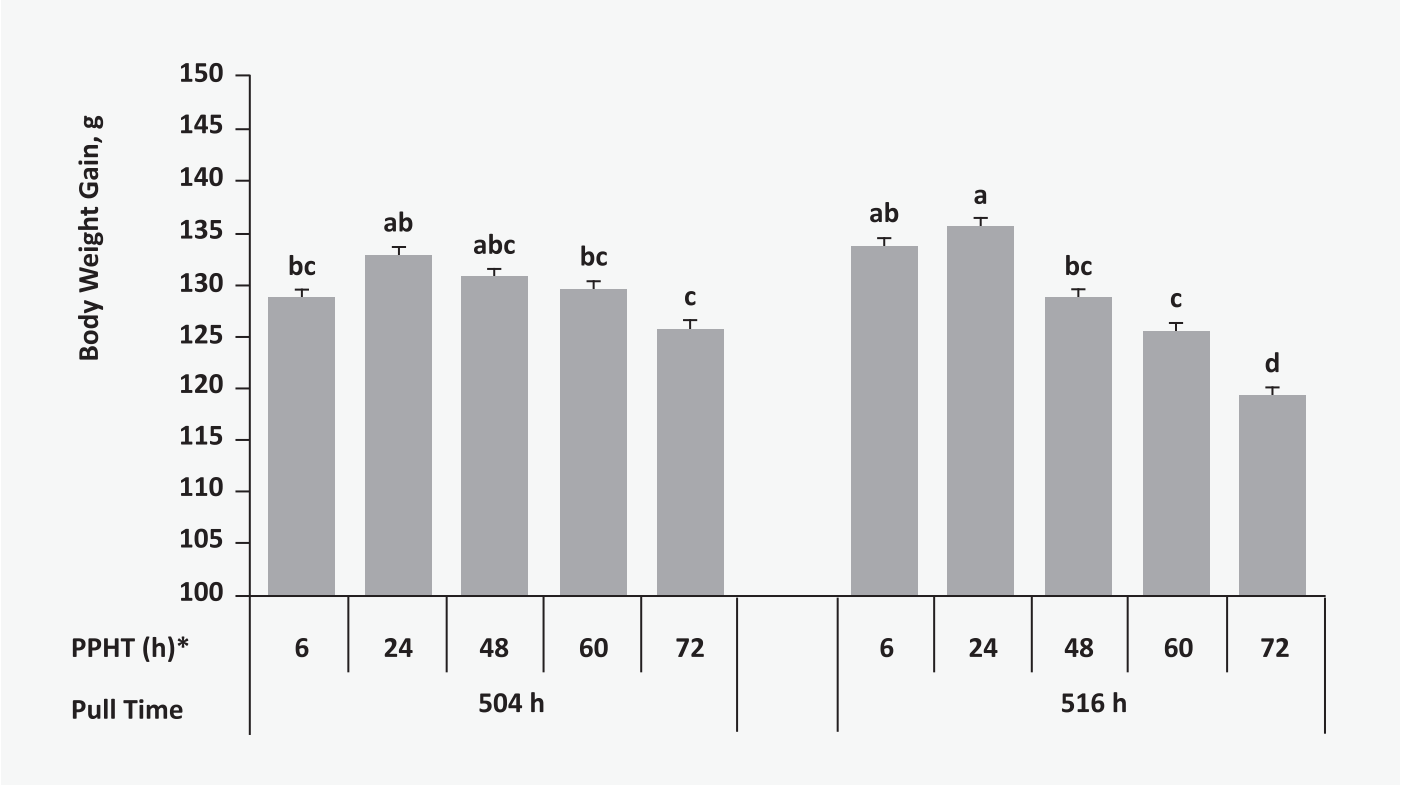
This study investigated the effects of the pull time and the preplacement holding time on yolk sac utilization, the crop filling, feeding behaviors and first-week BW and mortality. Broiler hatching eggs were obtained from a commercial broiler breeder flock of Ross 308 at 39 weeks of age and incubated in a commercial hatchery. In this trial, the chicks were removed from the hatcher at 504 h or 516 h of the incubation and counted into cardboard chick boxes containing 80 chicks each. The chicks were randomly distributed into 5 groups with different preplacement holding times under optimum conditions for each pull time group. The preplacement holding times were 6, 24, 48, 60, and 72 h from the pull time from the hatchers in the hatchery to placement in the broiler house on the farm, at which point the chicks were able to access feed and water. For the first week of the growing period after placement, a greater number of chicks (9600) were raised and 160 randomly selected chicks belonging to one of the 10 sub-treatment groups (5 chick preplacement holding time groups × 2 pulling time groups) were placed in each of 6 replicate floor pens (60 total pens) in a commercial broiler house. The feed and water access time did not influence yolk sac utilization, but the absolute or relative residual yolk sac (g, %) decreased linearly with the duration after the pull time ($P < 0.001$). Extended preplacement holding times were associated with a higher percentage of chicks with full crops at 3 hours after placement ($P < 0.001$). The lowest chick eating activity was observed in the 6 h group at 1, 3 and 8 h after placement in both pull time groups. Chick weight at placement was significantly reduced linearly with the duration after the pull time as expected ($P < 0.05$). The 24 h held chicks had a significantly higher BWG than 48 h and thereafter in the 516 h pull time groups, but this difference was only observed between 24 h and 72 h preplacement holding period in 504 h pull time groups (**Figure 1**; $P < 0.05$). Mortality within the first three days after placement increased only when the preplacement holding time was extended to 72 h ($P = 0.002$). Mortality during 4-7 d post-placement was not affected by the preplacement holding time at all, but the 72 h preplacement holding time group still had significantly higher cumulative mortality from 0 to 7 d after placement time ($P = 0.031$).

It can be concluded that there were no significant differences in average mortality, as a direct indicator of flock health welfare, up to and including a 60 h holding time under thermal comfort conditions, but a 72 h preplacement holding time increased mortality at 7 d of age after placement in both pull time groups. While the late pull time (516 h) compared to the early pull time (504 h) during the preplacement holding time had a beneficial effect on BWG for shorter holding period, early pull time has an advantage for the longer preplacement holding period.

Keywords: preplacement holding time, residual yolk sac, body weight, mortality.



*PPHT: Preplacement holding time

[a-d]: BWG in chicks with different superscripts differs significantly ($P < 0.05$).

Figure 1. Body weight gain (BWG) of chicks from placement to first 7 d.

Alternative hatching systems with early access to feed and water; what do we see in the field

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For chicks, time between hatching and placement can increase up to 72 hours, including holding time in the hatcher, processing, and transport. In that timeframe exogenous food and water is not available. The amount of waiting time and the absence of feed and water during that timeframe gained attention and concern in relation to welfare in Europe. Currently, the Dutch Court prescripts a maximal period of 36 hours without feed and water before arrival at the farm.
This has led to the development of alternative hatching systems, with options to provide feed and water during hatching at the hatchery (early feeding-systems), or to transport hatching eggs around day 18 of incubation to the farm, where the chicks will hatch (on-farm hatching systems).
The first impression of the benefit in providing chicks with early access to feed and water is promising in relation to chick body weight, yolk utilization, intestinal development, immune system development, and potential growth performance. However, the potential benefits might not be solely dependent on the concept of early feeding itself. Furthermore, the issue of increased welfare gained by early feeding is not easy to quantify.
Various early feeding- and on-farm hatching systems can nowadays be seen in the field. Feeding chicks in an early stage by an alternative hatching system, holds practical challenges in planning, capacity, technical issues, and transport. In addition, issues on cost-effectiveness, farm management, and planning the right day of delivery are topics that need consideration. Both systems require substantial long-term investments and therefore, investing in a system needs to be a well-considered choice. An investment in a certain system, has financial consequences and preferably needs to be returned. Improved technical results or better marketing price makes the investment feasible, however this is not always confirmed.
Although the necessity of proving feed and water during hatching might be questionable from a chick point of view, a positive attitude toward alternative hatching systems is present in the sector. The future for an early feeding- or on-farm hatching system might be found in the possible contribution in antibiotics reduction and the awareness that welfare, also regarding improved management at the hatchery as well as on the farm, can contribute to an improved technical performance.

Keywords: incubation, early feeding, on-farm hatching.

Effect of eggs pre-incubation on hatchability and physiological response of ducklings

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Pre-incubation, i.e. temporarily heating eggs, is one of the methods of improving the hatchability of prolonged storage of eggs. It seems interesting how this treatment depending on the age of the parental flock (APF) affects hatchability and selected biochemical blood parameters of ducklings.

Pekin duck hatching eggs (SM3, Cherry Valley) from three flocks at age: 28, 47 and 120 weeks (APF28, APF47 and APF120, respectively), stored for 21 days at T 16±1°C and RH 75±5%, divided into groups (378 eggs/group/flock): control and pre-incubated. Pre-incubated eggs were heated to 34°C/2h; 28°C/6h; 34°C/10h on the 5, 7 and 14 storage's day, respectively. Eggs were incubated in the setter S384 and the hatcher H192 (Sommen Incubators) in the commercial hatchery (E.G.G Ltd, Wieszowa, Poland). The one-day ducklings (n = 20 eggs/flock/group) were sampled and the blood plasma concentration of thyroxine, triiodothyronine, adrenaline, noradrenaline, dopamine, corticosterone and insulin were determined by radio immune assay. The influence of pre-incubation and APF on these biochemical parameters were examined with the two-way ANOVA, and Tukey's post-hoc test.

Pre-incubation did not improve the hatchability and quality of ducklings. In the pre-incubated in comparison to the control group, in APF28 the thyroxine concentration was about 6-fold lower ($P < 0.001$), but triiodothyronine concentration was not different, while in APF47 adrenaline concentration was about 2-fold lower ($P < 0.05$). In APF28 and APF47, noradrenaline in pre-incubated compared to control group was lower by c.a. 25% ($P<0.05$), while corticosterone was higher by 19% ($P = 0.10$) and 27% ($P <0.05$), respectively. On the other hand, the dopamine concentration in control (2.61 ± 0.676 ng/mL) was twice higher than in pre-incubated ducklings ($P = 0.002$), but only in APF47. The insulin concentration was not affected by the pre-incubation treatment ($P = 0.752$), however decreased with APF ($P <0.05$).

The reaction of hatched ducklings to the pre-incubation treatment of prolonged stored eggs may vary depending on the age of the reproductive flock.

Keywords: domestic duck, storage eggs, thyroid hormones, corticoids

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