## focus on RESEARCH

Focus on Research sponsored by the Incubation and Fertility Research Group (IFRG)

### Effect of the pipping rate and hatching nature on the development of artificially incubated ostrich chicks

Data for this study was collected from the commercial, pair-bred ostrich flock on the Oudtshoorn Research Farm, South Africa. Data was 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 the 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 0.85kg to 1.11kg, 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.

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### Effect of egg turning completion time during incubation on embryonic mortality and hatchability of broiler hatching eggs

The effect of turning duration and frequency during incubation has been widely investigated. However, little is known about the effects of turning completion time on hatchability. Furthermore, it is unclear what optimum turning completion time is required to see the benefits of egg turning. In this study, the effects of turning completion 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 3d 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 completion time to one side of each trolley was adjusted individually. The turning completion time was set to normal, and turning took less than one minute (<1 min=control) for one trolley; the other two trolleys were set for longer completion 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-minute 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 completion 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 the longer turning not having helped adjust the positioning of the embryo to achieve the correct formation to hatch. Rana Dişa<sup>1\*</sup>, Serdar Özlü<sup>2</sup>, Tülay Can<sup>1</sup>, and Okan Elibol<sup>2</sup>

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### Thermal imaging of the temperature of duck egg shells during incubation in a prototype hatching apparatus with an automatic sprinkling system

This study aimed 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/

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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 analysed with FLIR Tools+ to compare heat emission from the eggs in 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 the less-cooled one was on the outer edge of the tray (EST 37.2±0.30°C). However, the eggs in the upper levels were more strongly cooled closer to the medial edge. On these trays, cooling was slightly lower but more evenly

distributed (36.8 $\pm$ 0.23°C) compared to the lower ones (35.7 $\pm$ 0.83°C).

The modifications of incubation technology, despite some imperfections, increased 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 in the incubation process. Agnieszka Lisowska-Lis<sup>+\*</sup>, Aleksandra

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## Pullet hatchability and quality in hy-line brown laying lines: a data analysis

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  $\leq$ 6d were noted as "Fresh" eggs, while ES  $\geq$ 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 oF; the process took 9 hours with 3 hours of egg cooling. Multiple linear regression was used when no interaction effect

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### **Avian twins**

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) a 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 multiple 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 even though both oocytes are usually fertilised, and twins can survive 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 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 MTE development is stimulated more by environmental conditions than by genetic factors.

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(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 minimising 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\*FA2 -0.005\*FA\*ES - 0.008\*ES2 (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. Edgar O. Oviedo-Rondón<sup>1\*</sup>, Caleb M. Marshall<sup>1</sup>, and Daniel Valbuena<sup>2</sup> <sup>1</sup> Prestage Department of Poultry Science, North Carolina State University, Raleigh, NC, United States, Hy-line International, Des Moines, IA, United States \* Corresponding author: eooviedo@ncsu.edu