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

SPIDES – Turning Science into Practice

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 32˚C 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.

Dinah Nicholson, Aviagen, USA (retired)
Corresponding author: dinah.nicholson@klabang.co.uk

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

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 hypothesised 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 analysed using generalised 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 behavioural flexibility and are not as easily distracted or distressed as FW-only and deprived chicks.

Catharina M.H. Broekmeulen*, Yamenah Gómez, Sabine G. Gebhardt-Henrich and Michael J. Toscano
Centre for Proper Housing of Poultry and Rabbits, Animal Welfare Division, Veterinary Public Health Institute, University of Bern
*** Corresponding author: catharina.broekmeulen@unibe.ch**

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

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 Green legged 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 analysed 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.

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Effect of Nestmat Hygiene on Hatching Egg Quality and Chick Quality

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/m^2 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 randomised 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.

Katharina Geers[1]*, Frank van Merle[2], Gudo Mollen[2], Conny C. M. Maatjens[3], Carolyne Kemp[2] and Marcel Janssen[2]
[1] University of applied sciences Osnabrück, Faculty of Agricultural Sciences and Landscape, Oldenburger Landstraße 62, 49090 Osnabrück, Germany
[2] Aviagen EPI, Moerenstraat 89, 2370 Arendonk, Belgium
[3] CIQ Consultancy, Kerveldijk 1, 7223 KC, Baak, the Netherlands
*** Corresponding author: kgeers@aviagen.com**

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 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 normalised, and pesticide levels significantly decreased, suggesting reversible effects of pesticide exposure.

Skarlet Napierkowska[1]*, Pascal Froment[2], Artur Kowalczyk[3], Bożena Łozowicka[4], Piotr Kaczyński[4], and Agnieszka Partyka[1]
[1] Wroclaw University of Environmental and Life Science, Department of Reproduction and Clinic of Farm Animal Pl. Grunwaldzki 49 50-366 Wrocław;
[2] INRAE, UMR PRC, F-37380, Nouzilly, France;
[3] Wroclaw University of Environmental and Life Science, Institute of Animal Breeding, ul. Chełmońskiego 38C, 51-630 Wrocław,
[4] Institute of Plant Protection – National Research Institute, Laboratory of Food and Feed Safety, Bialystok, Poland
*** Corresponding author: skarlet.napierkowska@upwr.edu.pl**

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

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.

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

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

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°C 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.

Ahmet UÇAR[1], Mehmet Akif Boz[2] and Kadir Erensoy[3]*

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

[2] Yozgat Bozok University, Faculty of Agriculture, Department of Animal Science, Yozgat Türkiye

[3] Ondokuz Mayıs University, Faculty of Agriculture, Department of Animal Science, Samsun, Türkiye

*** Corresponding author: kadir.erensoy@omu.edu.tr**

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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.

Anne Pennings[1],[2]*, Jan Wijnen[2], Carla van der Pol[2], Jeroen Snijders[2] and Henry van den Brand[1]

[1] Adaptation Physiology Group, Wageningen University & Research, The Netherlands

[2] Research department, HatchTech Group, De Klomp, The Netherlands

*** Corresponding author: anne.pennings@wur.nl**