

Influence of light exposure during late incubation, early feeding, and litter access posthatch on the stress response and body weight gain in laying hen chicks

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Primary Audience: Producers, Poultry Manufacturing Industry, Researchers

SUMMARY

In-ovo sexing opens the door to on-farm hatching in the laying hen sector, but knowledge on the implications of different hatching system factors remains limited. This study addressed the influence of continuous light exposure during the last 3 d of incubation, posthatch feed and water access, and posthatch litter access on stress responsivity and body weight gain in laying hen chicks. Through a pilot experiment and two replicated experiments, we investigated the influence of these factors on physiological stress responsivity and body weight gain up to 8 wk of age. Short-term stress responsivity was evaluated by serum corticosterone and cloacal temperatures during processing procedures (e.g., sexing, sorting, vaccinating). Additionally, cumulative stress responsivity until 8 wk of age was assessed through corticosterone analysis in feather samples, and chick weights were recorded throughout. The presence of continuous lighting during the last 3 d of incubation affected stress responsivity, potentially by disrupting the circadian release of melatonin and thus corticosterone secretion. Immediate access to posthatch feed and water affected serum corticosterone levels, indicating a relationship between early feeding and stress responsivity. However, the underlying mechanisms remain unclear. Posthatch feed and water access did not have long-term effects on body weight gain, as fully deprived chicks were able to catch up within a day. Despite our study's exploratory nature, a complex relationship between stress responsivity and the hatching environment was shown, highlighting the need for future research to focus on disentangling and optimizing hatching system factors to minimize early life stress.

Key words: on-farm hatching, corticosterone, laying hen chick, hatching system factor

2024 J. Appl. Poult. Res. 33:100473

<https://doi.org/10.1016/j.japr.2024.100473>

DESCRIPTION OF PROBLEM

Hatchery-hatched chicks hatch in environments that are typically continuously dark (Archer and Mench, 2014, 2017) with high pathogen loads (De Gouw et al., 2017), high dust levels (De Gouw et al., 2017) and high noise levels (Hedlund et al., 2019). Due to a spread in hatching over a 24- to 48-h period (i.e., hatching window), some chicks might spend up to 2 d in hatchers before the hatch is pulled (Decuyper et al., 2001; Careghi et al., 2005). The pulling is then followed by human and/or automated handling that includes a range of processing procedures, such as sorting, sexing, vaccinations, and subsequent transportation to rearing farms (Hollemans et al., 2018; Hedlund et al., 2019; Giersberg et al., 2020). The delay in feed and water intake for hatchery-hatched chicks could therefore add up to 72 h (Sklan et al., 2000; Careghi et al., 2005; Willemsen et al., 2010; De Jong et al., 2019). Besides that, as a practical management procedure, hatchery-hatched chicks get access to litter substrate when they arrive to the rearing barn or after aviaries are opened (Brantsæter et al., 2017).

Adverse conditions during development are known to activate the hypothalamic-pituitary-adrenal (HPA) axis, resulting in non-essential physiological processes and behavior to be attenuated, as well as in adaptive programming of behavior (Zimmer et al., 2013; Bateson et al., 2014). Moreover, the level of exposure to adverse environmental factors during development could even have long-term effects on physiological and behavioral responses, by calibrating stress responsivity, and coping strategies (Monaghan and Haussmann, 2015). In fact, numerous studies, mostly in broilers, have reported that hatchery-hatched chicks are presented with environmental challenges that affect their performance and welfare. For instance, darkness during late incubation has been found to negatively affect the behavioral development and stress responsivity, and the ability to cope with stressors, due to a lack of

functional brain lateralization in broilers (Rogers, 2008; Archer and Mench, 2013, 2017). Also, limited litter access during rearing made adult laying hen more fearful compared to hens that had continuous access to litter during rearing (de Haas et al., 2014; Brantsæter et al., 2017). In addition, posthatch feed- and water deprivation has been found to negatively affect growth performance (Bigot et al., 2003; Gonzales et al., 2008; De Jong et al., 2017), gut development (Sklan et al., 2000; Gonzales et al., 2003), and disease susceptibility in chickens (Panda et al., 2015; Simon et al., 2015). Moreover, Hedlund et al. (2019) showed that hatchery-hatched laying hen chicks were more fearful of novelty, and had higher blood corticosterone levels after hatching, processing, and during the first weeks of life compared to unprocessed control chicks.

To address potential adverse effects of hatchery-hatched systems, two alternative hatching systems have been developed during the last decades. In the first alternative system, chicks are hatched in a hatchery and have immediate access to feed and water in the hatcher baskets but still need to be processed and transported to rearing farms. In the second alternative system, hatching eggs are transported on d 18 of incubation, and the chicks hatch on the litter of rearing barns (Van de Ven et al., 2009; De Jong et al., 2019; Giersberg et al., 2021; Souza da Silva et al., 2021). So far, several broiler studies have investigated how individual hatching system factors, such as light exposure in hatchers (Archer and Mench, 2013, 2014), and posthatch feed and water deprivation (e.g., Bigot et al., 2003; Gonzales et al., 2003; Van den Brand et al., 2010), affect stress responsivity. However, to our knowledge, only a limited number of studies have investigated the effects of individual hatching system factors and interactions between those factors on stress responsivity in laying hen chicks. In addition, the influence of litter substrate provision on early feeding during the hatching period has not been investigated. Therefore, our study aimed to investigate the impact

of three hatching system factors on aspects of HPA-axis reactivity in laying hen chicks up to an age of 8 wk. The objective of the first experiment was to identify which of the hatching system factors have an impact on the laying hen chick's acute and cumulative physiological and neuroendocrine responses by measuring body weight gain, cloacal temperature, and corticosterone levels in blood and feather samples. For the first experiment, which was a pilot experiment, we devised three treatments: light exposure during the last 3 d of incubation (L), immediate feed and water access (FW), and litter access posthatch (LIT) as hatching systems factors of interest. After completing the first experiment, we selected L and FW for a two-level factor study design for a more focused examination. The aim of the second experiment was to investigate the interaction of the selected hatching system factors. We hypothesized that chicks with exposure to light (i.e., L) and access to feed and water (i.e., FW) would show different acute and cumulative physiological stress responses compared to chicks that were kept in darkness during incubation and/or did not have access to feed and water posthatch.

MATERIAL AND METHODS

For the study we performed two experiments: experiment 1 (E1) was a pilot study, which ran from October until December 2021. The second experiment (E2) had 2 replicates, E2.1 and E2.2. The first replicate, E2.1, ran from June until August 2022, and the second (E2.2) from October until December 2022. The experiments were conducted at the Aviforum in Zollikofen, Switzerland.

Ethical Statement

All experimental procedures involving live animals were approved by the Veterinary Office of the canton of Berne, Switzerland (Cantonal license number BE54/2021).

Animals and Housing

The hatching eggs for all experiments were acquired from Prodavi SA (Switzerland). For

Table 1. Number of hatching eggs delivered, number of viable hatching eggs upon delivery, total number of female chicks, total number of male chicks, male to female ratio, hatchability, mortality around hatching, total number of chicks, number of experimental and focal animals during E1, E2.1 and E2.2.

Experiment	E1	E2.1	E2.2
Number of hatching eggs delivered	600	630	650
Number of viable hatching eggs upon delivery	583	613	628
Total number of female chicks	270	283	338
Total number of male chicks	302	319	282
Male to female ratio (%)	52.8 / 47.2	53.0 / 47.0	45.5 / 54.5
Hatchability (%)	95.3	95.6	95.4
Mortality around hatching (%)	4.7	4.4	4.6
Total number of chicks	572	602	620
Number of used experimental animals	243	268	282
Number of focal animals	75	64	64

each experiment, we used hatching eggs from a Super Nick hybrid-parent flock (H&N International GmbH, Cuxhaven, Germany) with a reproductive age of 32 to 33 wk. The number of hatching eggs, experimental animals, and focal animals per experiment is shown in [Table 1](#). A male to female ratio of 50:50, treatment, and embryonic mortality during the last 3 d of incubation were considered when deciding on the number of eggs within each pen. During all experimental procedures, the chicks were housed in an experimental barn without natural daylight. The behavioral tests took place in an adjacent room. The light schedule and barn climate settings were adjusted with each day or week of age according to the standard rearing management used by the Aviforum for white hybrids ([Supplementary 1](#)). During E1, the pens were set up in one row of seven pens and one row of nine pens. Fifteen out of the sixteen pens acted as treatment pens (3 pens/treatment group) with the sixteenth serving as a sick pen. The pen walls and doors were built with mesh frames (pen height: 206 cm) with plastic sheets attached as visual

barriers (height: 155 cm) (Supplementary 2). All pens could be individually accessed by a door. The pen interior consisted of a litter area (108 × 103 cm) with litter (provided from 7 d of age in the litter area of all pens) and a raised plastic tier (102 × 103 × 72.5 cm). Manure fell under the tier and was only removed at the end of the experiments. All pens were equipped with a ramp between the raised tier and litter area (116 × 24 cm, angle: 40°), one perch at tier level, one perch raised 5.5 cm above the tier (length: 100 cm, diameter: 4 cm), pen lights (height: 196 cm; SILOX, 22 W, 3,000 K (warm white), 2,400 lm, Once Animal Lighting, Signify, The Netherlands), drinker lines, and round feeders providing *ad libitum* feed. The light could be dimmed to a minimum of 15 lux by the animal caretakers or turned off as they were during incubation of the dark exposed treatments, according to flock management needs. During E2, one treatment pen was added on to the row of seven pens, resulting in seventeen pens in total. Sixteen out of the seventeen pens acted as treatment pens (4 pens/treatment group) with the seventeenth serving as a sick pen.

To divide the barn into a light and dark section and prevent light pollution during the treatment exposure period, tarpaulin sheets were attached to the barn ceiling and hung down to the ground. The dark section could only be accessed via a sluice (Supplementary 3). Multiple light intensity measurements were taken with a lux-meter (MAVOLUX 5032C/B USB, GOSSEN GmbH, Nürnberg, Germany) at the levels of the eggs (e.g., in the hatching tray), in the pens, and in the corridors. In the dark section, the readings gave <0.01 lux. In the light section, the readings gave 50 to 60 lux depending on the sensor position.

Climate loggers (HOBOWare, Onset Computer Corporation, Bourne, MA) were spread across six of the pens during each experiment. The climate loggers registered temperature and relative humidity in the barn at 1-h intervals.

The pens were designed to house up to 20 chicks during the study period, however the total number of chicks per pen varied due to differences in hatching rates (Table 2).

Table 2. Number of chicks per pen during E1, E2.1 and E2.2.

Pen	E1	E2.1	E2.2
1	14	20	14
2	18	19	20
3	14	12	20
4	18	16	20
5	17	18	20
6	15	13	16
7	13	15	20
8	16	14	18
9	16	14	19
10	16	17	15
11	18	20	12
12	15	18	18
13	15	20	20
14	18	15	14
15	18	19	19
16	-	18	17

Treatments

The hatching eggs were incubated in darkness during the first 18 d of incubation. Before candling, the egg trolleys were covered with fitted tarps when moved into the candling room. The eggs were kept in darkness during transportation and there was a sluice with tarps to enter the truck. Upon arrival at our site, the trolleys were covered with tarps and moved into the dark section of the barn, after which the eggs were assigned to one of the treatment groups. The treatment exposure period started as soon as the eggs were placed on the hatching trays in the pens on d 18 of incubation (Supplementary 2). The treatment exposure period ended when processing began with trained hatchery personnel to process the 1-day old chicks at our site. Thus, when light exposure was included as a treatment factor, the hatching eggs were exposed to artificial light (50–60 lux) upon placement in the pens on d 18 of incubation until chick were processed at 1 d of age. When light exposure was not included as a treatment factor, the eggs were kept in darkness (<0.01 lux). After hatching, the chicks were exposed to the same treatment conditions as described above until 1 d of age.

Experiment 1. Treatments consisted of continuous light exposure during the late incubation phase (d 18–21) (L), immediate feed and water access after hatching (FW),

Table 3. Treatment groups and treatment factors during E1.

Treatment	Light exposure	Feed and water access	Litter access
Fully enriched (ALL)	+	+	+
Fully deprived (CON)	-	-	-
Light (L)	+	-	-
Feed and Water (FW)	-	+	-
Litter (LIT)	-	-	+

immediate litter (i.e., wood shavings) access after hatching (LIT), fully enriched with all above mentioned treatment factors (ALL), or fully deprived (CON) (Table 3).

Experiment 2. Experiment 2 (E2) followed a 2×2-factorial design, with light exposure during the late incubation phase (L) and feed and water access after hatching (FW) as main treatment factors (Table 4). E2 had 2 replicate trials, E2.1 and E2.2. The light and feed and water access treatment factors for E2 were the same as described for E1.

On-Farm Hatching and Eggshell Temperature Monitoring

Upon arrival to the barn, the hatching eggs were parked in the dark section of the barn, after which groups of 40 hatching eggs were arbitrarily placed onto hatcher trays (67 × 57 cm; HatchTech B.V., Veenendaal, The Netherlands) within individual pens. The hatcher trays were elevated on bricks 20 cm above the raised plastic tiers (102 × 103 × 72.5 cm). The barn climate before hatching was set to an air temperature of 32°C, relative humidity of ≥ 30%, and air flow < 0.15 m/s. The barn climate was monitored in real-time at an interval of every 3 h, starting after the eggs were set until the last marked egg had hatched. The hatching was further monitored at this interval by measuring the

eggshell temperatures of three marked eggs per pen, where the objective was to maintain eggshell temperatures between 36°C and 38°C. Measurements were made by placing an infrared thermometer probe (MEDISANA TM 750, MEDISANA GmbH, Neuss, Germany) on the eggshell below the position of the air sac (i.e., on egg equator). The barn temperature was adjusted whenever the eggshell temperatures of the focal eggs were equal or below to 35.5°C. The climate settings could only be changed at barn-level. During E1, the barn temperature was adjusted once throughout the treatment exposure period. During E2.2, the barn temperature the barn temperature was adjusted four times throughout the treatment exposure period. Due to the darkness, we were not able to accurately assess the hatching window in the dark section of the barn, therefore we can only report the hatching window for at barn-level. The hatching window was approximately 24 h for E1, 29 h for E2.1 and 28 h for E2.2.

Chick Processing, Chick Quality Assessment and Break-Out Analysis

At 1 d of age, trained hatchery personnel came to the Aviforum site and processed the chicks according to commercial standards in the experimental room, which is attached to the barn. All chicks were subjected to processing procedures, which comprised of counting, sorting, sexing, grading, and vaccinating. The chicks were thereafter gathered in pen-specific crates to prevent mixing of treatment groups. All the processing steps combined, from catching to rehoming, took approximately 30 min in total. The female chicks remained in the barn and were vaccinated according to a commercial program (Supplementary 1). After all processing steps were completed, the chicks were relocated to a new home pen in a process that ensured that experimenters were blinded to the

Table 4. Treatment groups and treatment factors during E2.

Treatment	Light exposure	Feed and water access
Light and feed and water (L+/FW+)	+	+
Light (L+/FW-)	+	-
Feed and Water (L-/FW+)	-	+
Fully deprived (L-/FW-)	-	-

Table 5. Numbers of male chicks used for blood sample collection, weighing and temperature measurements at pre-processing, postprocessing, and recovery for E1, E2.1, and E2.2.

Experiment	Male chicks at preprocessing	Male chicks at postprocessing	Male chicks at recovery	Total number of male chicks
E1	30	30	30	90
E2.1	32	32	32	96
E2.2	32	34	36	102

treatment during behavioral testing. The surplus of female chicks was returned to the hatchery to be sold. For each experiment, random first grade male chicks (Table 5) were selected to stay on-site for postmortem blood collection after the processing procedures. The remaining surplus male chicks were returned to the hatchery to be humanely killed. In addition, a breakout analysis was performed to determine the underlying cause of unhatched eggs, such as embryonic mortalities, contaminations, and malformations. The breakout analysis revealed that several embryos perished before arriving on site, which led to a lower number of viable hatching eggs (Table 1).

Chick processing was chosen as a stressful early life event. It was not possible to include a control group without chick processing, because our research site operates according to commercial standards. Therefore, all day-old chicks had to be vaccinated for biosecurity reasons.

The study was designed according to the 3R principles and considered the male to female ratios and mortality during incubation. Therefore, we choose to collect samples from male chicks that were be hatched to acquire a certain number of female experimental animals for our experiment. This resulted in an overall reduced number of hatching eggs that needed to be incubated, and thus 1-day old male chicks that had to be transported back to the hatchery to be killed. It was assumed that the interaction between treatment factors and sex was negligible at the time of taking samples and physiological measurements.

Blood Sample Collection, Weighing and Measuring Cloacal Temperature of Male Chicks

The acute stress responsivity in chicks was assessed by collecting postmortem blood

samples for corticosterone analysis at three chick processing-associated times: (1) immediately before processing as a baseline (i.e., preprocessing), (2) postprocessing (i.e., immediately after all chicks were processed and approximately 4 h after preprocessing), and (3) recovery after 20 h (i.e., 24 h after preprocessing). During processing, 90 male chicks were marked with animal spray for E1, and 96 male chicks were marked for E2.1. Due to a miscount, 102 male chicks were marked for E2.2. Two marked (male) chicks were randomly selected per pen for blood collection at each of chick processing times. It was made sure that the entire process per pen—entering, catching plus handling for blood collection—took less than 180 seconds in duration (i.e., 2 chicks per pen per acute stress timepoint relating to chick processing), and a total duration of approximately 60 min to minimize confounding effects by the diurnal rhythm. After catching, the chicks were weighed (Mettler Toledo ICS425, Mettler-Toledo GmbH, CH-8606 Greifensee, Switzerland) and their cloacal temperatures were measured with an infrared thermometer (MEDISANA TM 750, MEDISANA GmbH, Neuss, Germany). Subsequently, the male chicks were stunned by concussive blow to the head, which was followed by cervical dislocation and immediate decapitation for blood sample collection ([Bündesamt für Lebensmittelsicherheit und Veterinärwesen, 2024](#)). As the male chicks were killed for blood samples, it was not possible to take longitudinal samples, i.e., multiple samples for the same individual chick.

Blood Serum Collection and Serum Corticosterone Analysis

Following blood collection, whole blood was left to clot for 30 min at room temperature and stored for 4 to 9 h at 3°C. Next, samples

were centrifuged (MiniSpin, Eppendorf SE, Hamburg, Germany), for 14 min at 2500 rpm x 1000 serum collected, and stored at -32°C .

A competitive enzyme immunoassay kit for corticosterone (High Sensitivity, AC-15F1, Immunodiagnostic Systems (IDS) Holdings PLC, Tyne & Wear, UK) was used according to the instructions of the producer's manual to measure serum corticosterone concentrations (analytical sensitivity 0.17 ng/mL). To validate the kit, we tested the recovery by spiking samples with corticosterone standard and found that recovery was approximately 100%. In addition, we made serial dilutions of the samples with assay buffer and checked for potential matrix effects (i.e., parallelity). The inclusion of serial dilutions was successful as we did not detect matrix effects.

A dilution of several samples in assay buffer was required either because the sample volume was not sufficient, or because the measured concentration of the undiluted samples was beyond the range of the standard curve. Therefore, the suitability of 1:4 and 1:10 sample dilutions were validated in three samples. Suitability of dilutions was approved by high parallelism at a CV of 4.5%. The intra and interassay CV of the corticosterone assay was 5.3 and 6.2%, respectively.

Weighing and Measuring Cloacal Temperatures of Female Chicks

At 1 d of age, after allocation to the final home pen, five female chicks per pen ($N = 75$ focal chicks) were randomly selected as focal animals for behavioral testing during E1. Four female focal chicks and four female companion chicks were selected per pen ($N = 64$ focal chicks) for behavioral testing during E2.1 and E2.2. Companion chicks were included during E2 to reduce fear and isolation stress during behavioral testing in focal chicks though no data of them was collected. The focal and companion chicks were tagged with flexible numbered leg rings (Flexiringe, Fieger AG, Untertuttwil, Switzerland) for individual identification. The leg rings were replaced by gradually larger leg rings with the same number at 1, 2, and 5 wk of age. The focal chicks were weighed (Mettler Toledo ICS425, Mettler-

Toledo GmbH, CH-8606 Greifensee, Switzerland) at 1, 7, 35, and 49 d of age during E1. During E2, both the focal and companion chicks were weighed at 1, 7, 14, 21, 28, 35, 42, and 49 d of age. The cloacal temperature of female chicks was measured in the same way as for male chicks at 1 d of age.

Behavioral Testing and Animal handling

Animal handling during behavioral tests is described in this paper as it could have resulted in physiological stress responses and thus the deposition of corticosterone in the feathers. The results of the behavioral testing during E1 and E2 are planned for publication elsewhere. All focal and companion chicks were subjected to carrying, catching, crating, transportation, waiting, habituation, and behavioral testing. For the multitasking test and retention test, each individual focal and companion chick underwent habituation to the test arena. For the step detour test, each individual focal animal underwent habituation to the test arena. Companion animals were excluded from the step detour test. In addition, all focal and companion animals were weighed. [Table 6](#) gives an overview of the different experimental phases during E1 and E2, and at what days of age the experimental animals were handled and approximate handling times per individual per experimental day. The habituation and behavioral tests are described in detail elsewhere ([Supplementary 4](#)).

Feather Sample Collection and Feather Corticosterone Analysis

At 8 wk of age, 10 plumage feathers (i.e., 20 mg) close to the neck and wings were collected from 60 focal and 60 nonfocal chicks ($n = 12$ focal chicks/treatment; $n = 12$ nonfocal chicks/treatment) during E1. During both E2.1 and E2.2, feathers were collected from 64 focal chicks and 64 companion chicks ($n = 16$ focal chicks/treatment; $n = 16$ companion chicks/treatment). The feathers were collected by cutting immediately above the base of the feather shaft.

Table 6. Overview of days of age (DOA) at which experimental animals were handled during different experimental phases, approximate handling times per individual during all the experimental phases and the total of days at which chicks were handled for focal chicks during E1 and focal and companion chicks during E2.

Experimental phase	Days of age during experimental phase (DOA)				Handling time / chick / day (in minutes)
	Experiment 1 (E1)		Experiment 2 (E2)		
	Focal chick	Focal chick	Companion chick	Companion chick	
Habituation to multitasking and retention test arena	3–7 DOA	3–9 DOA			30
Multitasking test	9 DOA	10 or 11 or 12 or 13 DOA			30
Retention test	10 DOA	If multitasking test at 10 or 11 DOA, then retention test at 14 DOA If multitasking test at 12 or 13 DOA, then retention test at 15 DOA			15
Habituation to step detour test arena	22–26 DOA	22–26 DOA	-		30
Step detour test	29–34 DOA	29–34 DOA	-		30
	43–48 DOA	43–48 DOA			
Weighting	1, 7, 35, 49 DOA	1, 7, 14, 21, 28, 35, 42, 49 DOA			15
Total of d on which chicks were handled	28 d	34 d		17 d	

Feather Corticosterone Analysis

An average feather mass of 25.1 mg from three to five feathers (range 16.0–28.0 mg) per individual was used to measure feather CORT. The feathers were weighed, and the length measured to the nearest millimeter (E1: feather mass 24.8 ± 1.9 mg, feather length 164.3 ± 17.5 mm; E2: feather mass 25.2 ± 1.4 mg, feather length 173.0 ± 20.1 mm). Before weighing and measuring the feathers, the calamus was removed. The feathers were placed in a 2 ml polypropylene microcentrifuge tube, cut into small pieces and ground to powder using a mixer mill (RETSCH MM400, Haan, Germany) at 30 Hz for 6 min with 10.3 mm metal balls. Then 1.5 ml of methanol was added to each tube and the tubes were placed in an ultrasonic bath for 30 min at room temperature and then incubated overnight at 50°C in a water bath. The methanol with the feather powder was pipetted into a new microcentrifuge tube, centrifuged at 14.000 rpm for 10 min and 1 ml of the supernatant was transferred to a glass centrifuge tube. The methanol was evaporated with nitrogen gas at 50°C and the extracts were stored in a freezer at –20°C until measurement. The extracted CORT was measured using a commercially available corticosterone ELISA kit (ENZO ADI-901-097, Farmingdale, NY) according to the instructions. To check for potential matrix effects (i.e. parallelism) for our samples in the assay, we made a five-point serial dilution (1:10–1:80) of the chicken pool with assay buffer. Dilutions between 1:10 and 1:30 are parallel to the standard curve and are accepted in the assay. The extracted corticosterone was resuspended in 330 μ l of assay buffer prior to ELISA analysis. A pool of chicken feathers was run in duplicate on each plate as an internal control (mean intra and interassay variation: 10.5 ± 5.7 %; 24.8 ± 1.1 %, respectively).

Statistical Analysis

The statistical analyses were carried out with R (R Core Team, Version 4.3.1) using RStudio (Rstudio Team, Version 2023.06.1+524). The dataset for E1 and E2.1/E2.2 were analyzed separately. E2.2 was a replicate experiment of E2.1,

therefore the datasets were combined and analyzed as E2. For both analyses, $P < 0.05$ was set as the limit for statistical significance. The response variables (body weight of male and female chicks, serum corticosterone concentration of male chicks, cloacal temperature of male and female chicks, and feather corticosterone concentration of female chicks) were classified as continuous responses and analyzed using linear mixed effects (package lme4; Bates et al., 2015). The response variables were the same in analyses of E1 and E2. Responses for E1 and E2 were fitted with a different linear mixed-effects model for each variable. The linear mixed-effects models for body weight, cloacal temperature, and serum corticosterone concentrations of male chicks included a two-way interaction between treatment (levels for E1: CON, ALL, L, FW, LIT; levels for E2: FW+/L+, FW+/L-, FW-/L+, FW-/L-) and processing time (levels: preprocessing, postprocessing, recovery) as fixed factors. The cloacal temperature in female chicks was measured only once, therefore only treatment was included as a fixed factor. The model for body weight of female chicks included a two-way interaction for treatment and age (4 levels for E1: 1 DOA (day of age), 7 DOA, 35 DOA, 49 DOA; 8 levels for E2: 1 DOA, 7 DOA, 14 DOA, 21 DOA, 28 DOA, 35 DOA, 42 DOA, 49 DOA) as fixed factors. The model for feather corticosterone concentrations included a two-way interaction for treatment and chick status (levels for E1: focal, nonfocal; levels for E2: focal, companion). For all above-mentioned linear models, pen was nested in treatment group as a random factor. For the analysis of E2, pen, and treatment group were nested in replicate experiment (E2.1 vs. E2.2) as a random structure for all the above-mentioned linear models. The model residuals were visually inspected for normal distribution with Q-Q plots or tested with the package DHARMA (Hartig, 2018). If the residuals were nonrandom, the raw data was transformed by the square-root function on the response variable and re-evaluated. The full model was reduced to the final model by stepwise backwards model reduction using likelihood ratio tests (LRT) for model comparisons. Subsequently, Tukey's *post hoc* tests were performed (package multicomp; Holthorn et al., 2008) to determine differences between levels of

the fixed factors. The *post hoc* test output was used to obtain estimates and standard errors. For both E1 and E2, the data for serum corticosterone concentrations and body weight of female chicks required square-root transformations. The model estimates for serum corticosterone concentrations of male chicks, and body weight of female chicks were back-transformed for presentation of results.

RESULTS AND DISCUSSION

The experiments were aimed at assessing the effects of various hatching system factors on aspects of the physiological stress responsivity and body weight gain in laying hen chicks up to an age of 8 wk. In the experiments, we assessed the impact of continuous light exposure during the last 3 d of incubation, immediate feed and water access posthatch, and litter access posthatch, and their interactions.

Serum Corticosterone Levels in Male Chicks

The analysis of serum corticosterone levels revealed that the physiological stress response in newly hatched male chicks was affected by an interaction between treatment and processing time (E1: $X^2 = 24.577$, $df = 8$, $P = 0.002$; E2: $X^2 = 536.92$, $df = 6$, $P < 0.001$, respectively) (Figure 1). However, the stress response deviated from the typical adaptive pattern, where corticosterone levels rise in response to acute stressors and return to baseline levels after removal of those stressors (Cockrem, 2007; Scanes, 2016; Majer et al., 2023). Serum corticosterone levels were higher at preprocessing compared to postprocessing in ALL, CON, and LIT chicks during E1 (2.9 ± 0.5 ng/ml; 5.3 ± 0.5 ng/ml; 2.7 ± 0.5 ng/ml, respectively). Additionally, serum corticosterone levels were higher at preprocessing compared to recovery in CON and LIT chicks during E1 (4.1 ± 0.5 ng/ml; 5.9 ± 0.5 ng/ml, respectively). The same pattern was also observed during E2, where serum corticosterone levels were higher at preprocessing compared to recovery in fully deprived chicks (1.6 ± 0.4 ng/ml) (Figures 1 and 2). During our experiments, the hatching eggs were exposed to either continuous light or

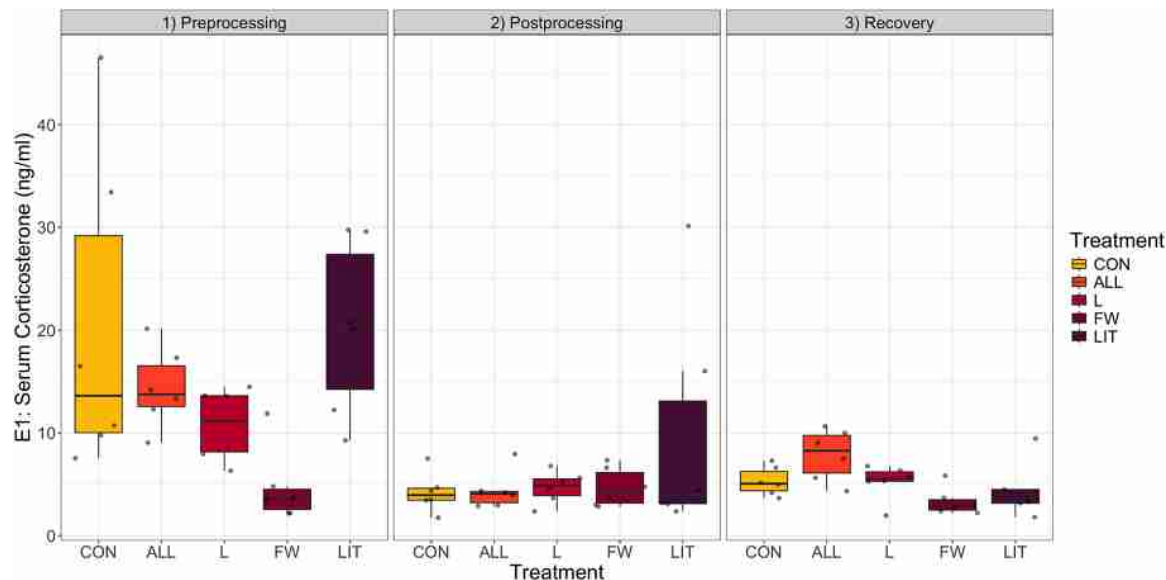


Figure 1. The plot shows the serum corticosterone levels of male chicks at the three acute stress timepoints around chick processing: (A) preprocessing, (B) postprocessing, and (C) recovery of Experiment 1 (E1). The abbreviations on the x-axis represent the treatment groups during E1, with CON for fully deprived chicks, ALL for fully enriched chicks, L for chicks that were exposed to continuous light during the late incubation phase (d 18–21), with FW for chicks that had immediate access to feed and water after hatching and LIT for chicks that had immediate access to litter after hatching. The serum CORT levels revealed an interaction between treatment and handling time ($X^2 = 24.577$, $df = 8$, $P = 0.002$). The serum CORT levels were higher at preprocessing compared to postprocessing in ALL, CON, and LIT chicks (2.9 ± 0.5 ng/ml; 5.3 ± 0.5 ng/ml; 2.7 ± 0.5 ng/ml, respectively). Additionally, serum CORT levels were higher at preprocessing compared to recovery in CON and LIT chicks (4.1 ± 0.5 ng/ml; 5.9 ± 0.5 ng/ml, respectively).

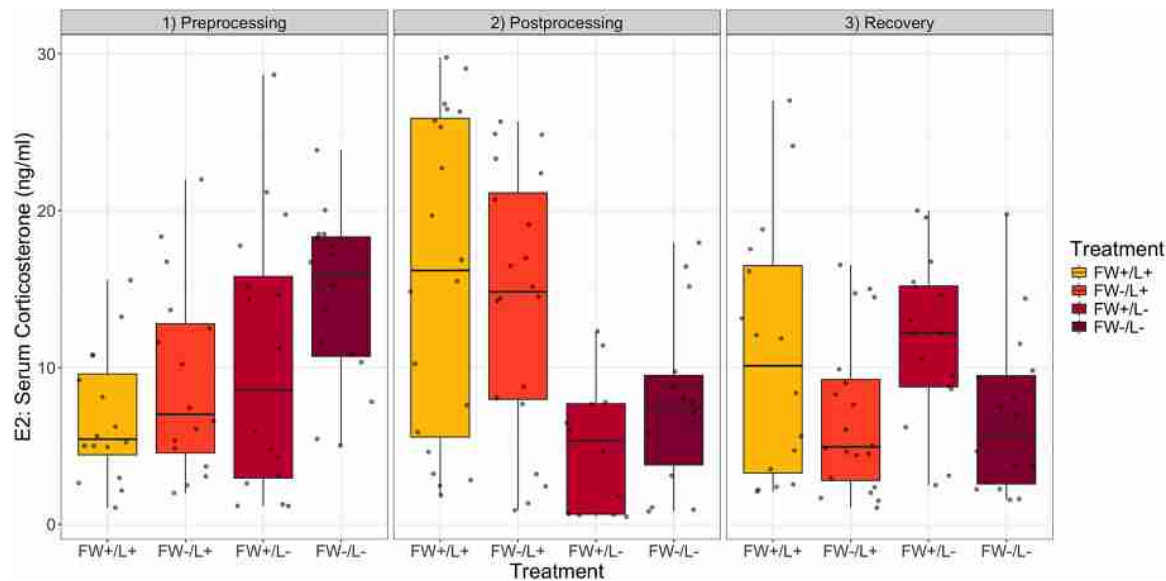


Figure 2. The plot shows the serum CORT levels of male chicks at the three acute stress timepoints around chick processing: (A) preprocessing, (B) postprocessing, and (C) recovery in Experiment 2 (E2). The abbreviations on the x-axis represent the treatment groups during E2, with light exposure during the late incubation phase (L+) and feed and water access after hatching (FW+). The serum CORT levels revealed an interaction between treatment and processing time ($X^2 = 536.92$, $df = 6$, $P < 0.001$). FW-/L- chicks had higher serum CORT levels than FW+/L+ chicks at preprocessing (1.7 ± 0.4 ng/ml). FW+/L+ chicks had higher CORT levels at postprocessing compared to preprocessing (1.4 ± 0.3 ng/ml). FW+/L- chicks had lower serum CORT levels at postprocessing compared to FW+/L+ and FW-/L+ (2.7 ± 0.4 ng/ml; 2.5 ± 0.4 ng/ml, respectively). FW+/L- and FW-/L+ chicks had lower serum CORT levels at compared to postprocessing (1.7 ± 0.3 ng/ml; 1.0 ± 0.3 ng/ml, respectively). FW-/L- chicks showed higher serum corticosterone at preprocessing compared to recovery (1.6 ± 0.4 ng/ml).

darkness during late incubation, whereas under natural conditions, eggs are exposed to light intermittently under natural brooding conditions (Rogers, 1995; Buschmann et al., 2006). Potentially, the lack of intermittent light exposure during our experiments might have affected the circadian rhythm, as occasional dark periods during embryonic development are crucial for the establishment of circadian rhythms through melatonin secretion (De Jong et al., 2001; Zeman et al., 2004). Additionally, melatonin modulates the physiological stress response by inhibiting an increase in corticosterone (Saito et al., 2005). Our unexpected findings about the corticosterone release might therefore reflect that continuous light exposure and darkness disrupted the rhythmicity of melatonin secretion, and thereby HPA-activity and corticosterone secretion, which subsequently led to the higher baseline levels found at preprocessing compared to postprocessing and recovery. The interpretation is also supported by our findings during E2, where FW-/L- chicks had higher serum corticosterone levels than FW+/L+ chicks at pre-processing (1.7 ± 0.4 ng/ml). In support of our findings, a broiler study reported reduced corticosterone levels after crating in light-incubated chicks compared to dark-incubated chicks (16 h light: 8 h dark), (Özkan et al., 2012). Another broiler study that compared different lighting schedules, found lower corticosterone levels after crating in 12 h light-incubated chicks (12 h light: 12 h dark) compared to chicks that were exposed to shorter daylight periods (1 h light: 23 h dark, 6 h light: 18 h dark) (Archer and Mench, 2013).

Moreover, it is assumed that all chicks had the same experience prior to preprocessing aside from the treatment they were exposed to. Thus alternatively, the greater serum corticosterone levels in CON and LIT chicks at preprocessing compared with postprocessing and recovery could be attributed to the sudden switch from darkness to light shortly before blood collections started at preprocessing. The rapid transition from darkness to light could have triggered acute physiological and behavioral response in dark-incubated chicks, which might have resulted in a more pronounced increase in CORT.

During E2, only the corticosterone response of FW+/L+ chicks conformed to the expected

pattern with greater postprocessing values compared to preprocessing (1.4 ± 0.3 ng/ml), similar to reports by Hedlund et al. (2019). This suggests that fully enriched chicks were able to develop an appropriate stress response in terms of glucocorticoid concentrations (Figure 2). The stress response in FW+/L+ chicks (and the corresponding lack of a difference in the other groups) might be explained by the chicks hatching under conditions with comparable elements to the natural hatching environment, including access to feed and water after hatching, and more space to move. However, it is unclear how the combination of these environmental conditions affects the development of the physiological stress response in chicks.

Serum corticosterone levels were expected to be lower at postprocessing in light-incubated chicks in comparison to dark-incubated chicks because the lights for the latter were on for approximately 4 h (i.e., immediately before at preprocessing). De Jong et al. (2001) showed that the corticosterone concentration reaches its circadian peak levels after 4 h of light exposure. Animal handling could have affected the serum corticosterone release by introducing additional stressors, although, the chicks might have been habituated to our presence and activity in the barn due to the monitoring during hatching. Unexpectedly, lower postprocessing serum corticosterone levels were found in FW+/L- chicks compared to FW+/L+ and FW-/L+ (2.7 ± 0.4 ng/ml, 2.5 ± 0.4 ng/ml, respectively), indicating that immediate feed and water access posthatch might have affected CORT. However, it is unclear which mechanisms are involved and if early feeding affected stress sensitivity in chicks with immediate access to feed and water posthatch.

Focusing on the recovery period, serum corticosterone levels were anticipated to be similar to the preprocessing serum corticosterone levels. This stress responsivity pattern was only observed for FW+/L- and FW-/L+ chicks during E2, as they showed lower serum corticosterone at recovery compared to postprocessing (1.7 ± 0.3 ng/ml; 1.0 ± 0.3 ng/ml, respectively). In contrast, fully deprived chicks showed higher serum corticosterone at preprocessing compared to recovery (1.6 ± 0.4 ng/ml). It might be possible

that these chicks needed a longer time to habituate to the continuous lighting schedule that started at preprocessing and/or changes in the home pen environment compared to chicks that were not deprived, which in turn could have affected stress responsivity.

Overall, the serum corticosterone levels did not show the anticipated increase after chick processing but corresponded with serum corticosterone levels that have been reported in other studies that focused on 1-day old chicks (Ericsson and Jensen, 2016; Hedlund et al., 2019). Therefore, it is important for future work to explore whether the induction of multiple stressors, in this case the chick processing steps, have additive effects including habituation or sensitization in chicks.

One of the limitations of our study is that we were not able to perform within- individual comparisons at different processing times since the male chicks were killed for blood samples, while both basal and “stress-induced” elevations in corticosterone levels are subject to within- and between-individual variation (Littin and Cockrem, 2001).

Body Weight in Male Chicks

Body weight demonstrated an interaction between treatment and processing time in (male) chicks during E1, which aligns with our hypothesis ($X^2 = 36.275$, $df = 14$, $P < 0.001$). Notably, weight gain was only observed in the fully deprived chicks (CON), with a tendency for higher body weights at recovery compared to preprocessing (4.0 ± 1.2 g), and higher body weights at recovery compared to postprocessing (4.73 ± 1.2 g) (Figure 3). Feed and water deprivation and darkness during late incubation seemed to impact preprocessing body weight. However, these effects only appear to be short-lived since fully deprived chicks did not differ in weight by recovery. The chicks had access to feed and water for 20 h before weighing at recovery, raising the possibility that the deprivation period was not severe enough since all chicks were provided with feed and water after postprocessing, which might have allowed fully deprived chicks to adapt within this 20-h time-frame (i.e., from postprocessing to recovery).

Peer-reviewed literature on early feeding is limited, where some studies in broiler chicks suggest that there is a relationship between hatching time, early feeding behavior, and feed intake (Nielsen et al., 2010; De Jong et al., 2016; Jessen et al., 2021). Unfortunately, we could not record individual hatching times and feeding behavior during our study.

For E2, the body weights of male chicks were affected by processing time but not by treatment ($X^2 = 29.735$, $df = 12$, $P < 0.01$), which likely reflects daily body weight gain (Figure 4). The feed and water deprivation treatment might not have been severe enough to cause treatment differences in body weight gain during our study. Alternatively, laying hen chicks may respond differently to immediate feed and water access posthatch, and feed and water deprivation, potentially due to different metabolic demands compared to broilers. Several studies examining on-farm hatching in broilers demonstrated that immediate feed and water access posthatch results in higher body weights in on-farm hatched chicks compared to hatchery-hatched chicks (Van de Ven et al., 2009; De Jong et al., 2019; Jessen et al., 2021), as well as hatchery-fed chicks (Souza da Silva et al., 2021; Molenaar et al., 2023). Therefore, it is important to consider genetic differences between laying hens and broilers in the context of on-farm hatching and early feeding studies.

Cloacal Temperature in Male and Female Chicks

The analysis of cloacal temperatures in male chicks did not reveal any effects of treatment or processing time for E1 ($P > 0.05$). Similarly, there were no effects of treatment on cloacal temperatures in female chicks for E1 ($P > 0.05$). However, for E2, cloacal temperatures in male chicks were found to be influenced by processing time but not by treatment ($X^2 = 25.034$, $df = 11$, $P < 0.01$), exhibiting higher temperatures at both preprocessing and postprocessing compared to the recovery ($0.5 \pm 0.1^\circ\text{C}$; $0.3 \pm 0.1^\circ\text{C}$, respectively) (Figure 5). The elevated cloacal temperatures at preprocessing and postprocessing might indicate a physiological stress response to various environmental

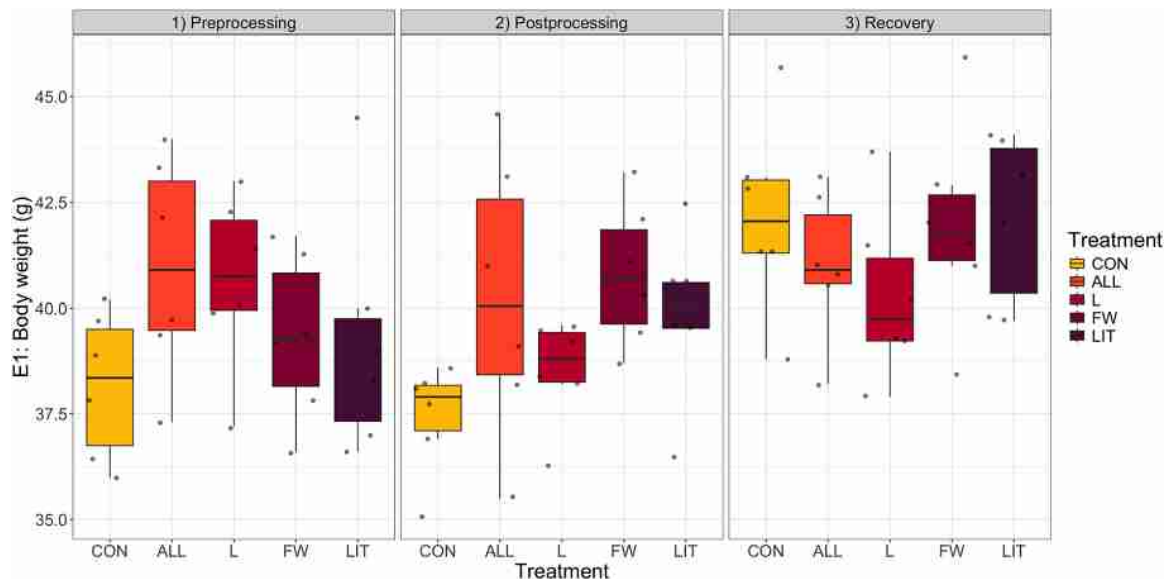


Figure 3. The plot shows the body weights of male chicks at the three acute stress timepoints around chick processing: (A) preprocessing, (B) postprocessing, and (C) recovery. The abbreviations on the x-axis represent the treatment groups during E1, with CON for fully deprived chicks, ALL for fully enriched chicks, L for chicks that were exposed to continuous light during the late incubation phase (d 18–21), with FW for chicks that had immediate access to feed and water after hatching and LIT for chicks that had immediate access to litter after hatching. Body weights demonstrated an interaction between treatment and processing time ($X^2 = 36.275$, $df = 14$, $P < 0.001$). Weight gain was only observed in the fully deprived chicks (CON), with a tendency for higher body weights at recovery compared to preprocessing (4.0 ± 1.2 g), and higher body weights at recovery compared to postprocessing (4.73 ± 1.2 g).

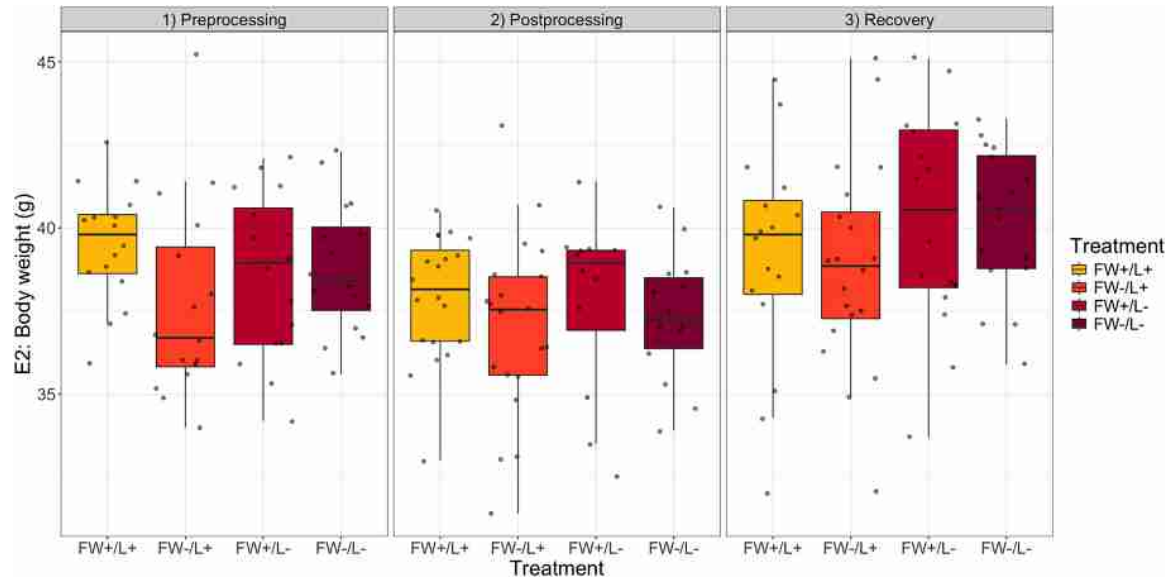


Figure 4. The plot shows the body weights of male chicks at the three acute stress timepoints around chick processing: (A) preprocessing, (B) postprocessing, and (C) recovery. The abbreviations on the x-axis represent the treatment groups during E2, with light exposure during the late incubation phase (L+) and feed and water access after hatching (FW+). The body weights of male chicks were affected by processing time but not by treatment ($X^2 = 29.735$, $df = 12$, $P < 0.01$).

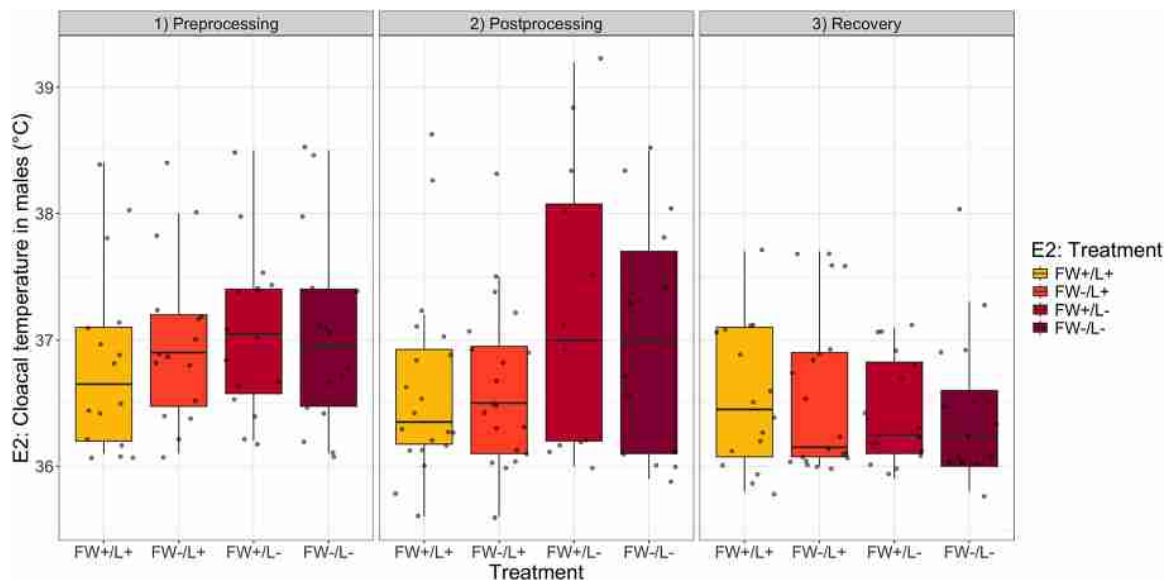


Figure 5. The plot shows the cloacal temperatures of male chicks at the three acute stress timepoints around chick processing: (A) preprocessing, (B) postprocessing, and (C) recovery. The abbreviations on the x-axis represent the treatment groups during E2, with light exposure during the late incubation phase (L+) and feed and water access after hatching (FW+). The cloacal temperatures were influenced by processing time but not by treatment ($X^2 = 25.034$, $df = 11$, $P < 0.01$), exhibiting higher temperatures at both preprocessing and postprocessing compared to the recovery ($0.5 \pm 0.1^\circ\text{C}$; $0.3 \pm 0.1^\circ\text{C}$, respectively).

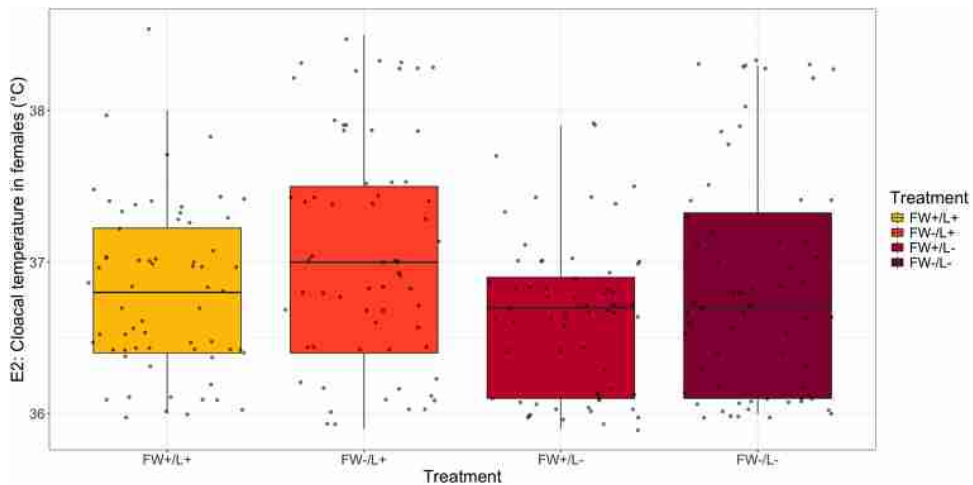


Figure 6. The plot shows the cloacal temperatures of female chicks during E2. The abbreviations on the x-axis represent the treatment groups during E2, with light exposure during the late incubation phase (L+) and feed and water access after hatching (FW+). The cloacal temperatures were affected by treatment ($X^2 = 7.8845$, $df = 3$, $P < 0.05$), with higher cloacal temperatures for FW-/L+ chicks compared to FW+/L- chicks ($0.4 \pm 0.2^\circ\text{C}$).

changes, including the transition from darkness to light for chicks in the dark section of the barn, as well as human handling in all chicks. The cloacal temperatures in female chicks during E2 were affected by treatment ($X^2 = 7.8845$, $df = 3$, $P < 0.05$), with higher cloacal temperatures for FW-/L+ chicks compared to FW+/L- chicks ($0.4 \pm 0.2^\circ\text{C}$) (Figure 6). Besides affecting the melatonin rhythm which is associated with body temperature regulation (Yalçın et al., 2022), lighting has been reported to stimulate higher activity (i.e., locomotion) in chicks (Olanrewaju et al., 2006; Wichman et al., 2021), which in turn could have elevated their cloacal temperatures (Yahav, 2015).

Body Weight Gain in Female Chicks

Body weights in female chicks demonstrated an interaction between treatment and week of age during E1 and E2 ($X^2 = 1,444.5$, $df = 9$, $P < 0.001$; $X^2 = 9548.5$, $df = 7$, $P < 0.001$, respectively), reflecting the weekly body weight gain for female chicks within all treatment groups. Despite this interaction, the body weight gain did not differ between treatments. Our findings might indicate that effects of the treatment exposure period on body weight gain were short-lived. Simon et al. (2014), for instance, reported that early-fed laying hens had higher body weights (i.e., up to 35 d of age) compared

to hens that were deprived for 72 h posthatch. However, laying hen chicks in the study by Simon et al. (2014), did not hatch on-farm and transport might have affected body weights, as well. Witjes et al. (2022), for example, reported higher body weights in on-farm hatched laying hen chicks up to 75 d compared to chicks that were resource-deprived and underwent 8 h of transportation.

Feather Corticosterone Levels in Female Chicks

The analysis of feather corticosterone in female chicks revealed an interaction between chick status (i.e., focal or not) and treatment for E1 ($X^2 = 31.888$, $df = 9$, $P < 0.01$). Notably, differences in feather corticosterone levels were only observed in L+ chicks, with higher levels in non-focal chicks compared to focal chicks (0.6 ± 0.2 pg/mm) (Figure 7). Possibly, the combination of human handling and grape rewards after testing was perceived as positive by the focal chicks, leading to habituation and the testing becoming an enrichment (Jones, 1993; Jones and Waddington, 1993; Hems-worth et al., 1994). The analysis of feather corticosterone levels in female chicks did not reveal any effects of chick status (i.e., focal or companion) or treatment for E2 ($P > 0.05$). During E2, we collected feathers from focal

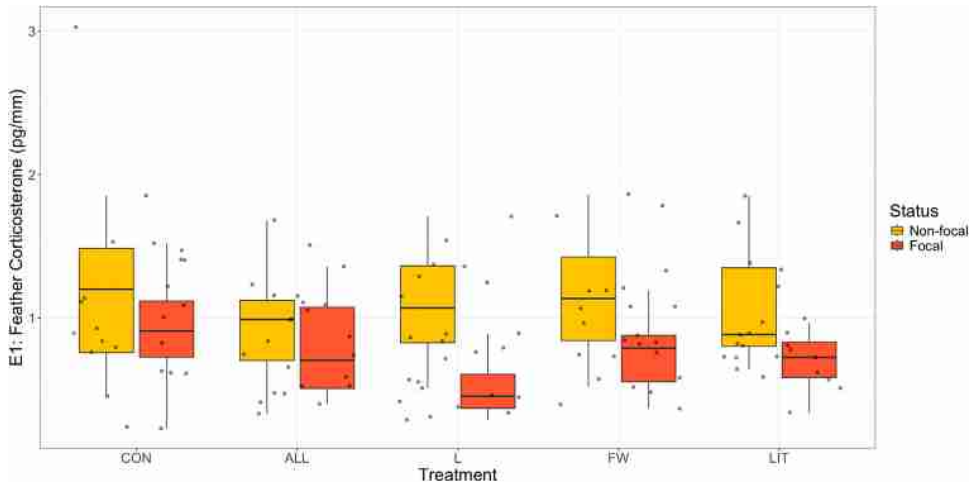


Figure 7. The plot shows the feather CORT levels of female chicks during E1. The abbreviations (CON, ALL, L, FW, LIT) on the x-axis represent the treatment groups during E1, with CON for fully enriched chicks, ALL for fully deprived chicks, L for chicks that were exposed to continuous light during the late incubation phase (d 18–21), with FW for chicks that had immediate access to feed and water after hatching and LIT for chicks that had immediate access to litter after hatching. Feather CORT levels were affected by an interaction between chick status and treatment ($X^2 = 31.888$, $df = 9$, $P < 0.01$). Nonfocal chicks in the L treatment group had higher feather CORT compared to focal chicks (0.6 ± 0.2 pg/mm).

chicks and companion chicks. It is possible that the amount of handling between focal chicks and companion chicks was not large enough to show differences in status for feather CORT levels as when comparing focal and nonfocal chicks during E1 (Table 6). CORT is only deposited into feathers during their growth phase (Jenni-Eiermann et al., 2015), which is why we collected feathers at 8 wk of age. After this point, molting would result in new feather growth, preventing us from accurately linking CORT levels to the experimental period (Lucas et al., 1972).

CONCLUSION AND APPLICATIONS

Our study investigated the impact of various hatching system factors on the physiological stress responsivity and body weight gain of laying hen chicks.

1. Continuous light exposure and darkness during the perinatal period could potentially affect the development and functionality of the stress responsivity in chicks by influencing circadian rhythms.
2. Immediate access to feed and water post-hatch seemed to influence serum CORT

levels, which suggests a link between early feeding and stress responsivity. However, it is unclear which underlying mechanisms are involved.

3. Treatment had a short-lived impact on body weights with fully deprived chicks already gaining weight by recovery. No long-term effects of treatment on body weight gain were found. While early feeding sounds promising, genetic differences between laying hen and broilers should be taken into consideration.
4. Our findings underscore the complexity of the dynamics between stress responsivity and the environment during the perinatal period. We believe that our study helped to determine future research directions and want to emphasize that hatching system factors should be disentangled further.
5. The limitations of our study were that we were not able to perform within individual comparisons of serum corticosterone at different processing times nor were we able to quantify posthatch feeding behavior and the hatching window. Therefore, observations on posthatch feeding behavior and the hatching window should be included in future studies.

DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGMENTS

The authors want to thank the Aviforum staff for their excellent animal care.

We are grateful to Klara Grethen, Alex Johny, Daniel Lienhardt, Masha Marincek, Amina Niederberger, Abdulsatar Rahman, Josie Siegel, and all student helpers for their assistance during behavioral testing and the collection of physiological samples. Thanks to Jan Büchler for designing the barn lay-out. Many thanks to Thomas Heinzel for building the pens and testing apparatuses. We also want to thank Andreas Suter and his team for assisting us during the chick processing procedures. In addition, we would like to thank Susanne Vonk and Gust Fleerackers for their technical support during the hatching. The expert laboratory work of Chantal Philipona, Veterinary Physiology, to measure serum corticosterone is very much appreciated. This project received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No. 812777.

Data availability: The data and code for this study are available at <https://OSF.IO/XZQRN/>.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.japr.2024.100473.

REFERENCES

Archer, G. S., and J. A. Mench. 2013. The effects of light stimulation during incubation on indicators of stress susceptibility in broilers. *Poult. Sci.* 92:3103–3108.

Archer, G. S., and J. A. Mench. 2014. Natural incubation patterns and the effects of exposing eggs to light at various times during incubation on post-hatch fear and stress responses in broiler (meat) chickens. *Appl. Anim. Behav. Sci.* 152:44–51.

Archer, G. S., and J. A. Mench. 2017. Exposing avian embryos to light affects post-hatch anti-predator fear responses. *Appl. Anim. Behav. Sci.* 186:80–84.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Linear mixed-effects models using {lme4}. *J. Stat. Softw.* 67:1–48.

Bateson, P., P. Gluckman, and M. Hanson. 2014. The biology of developmental plasticity and the predictive adaptive response hypothesis. *J. Physiol.* 592:2357–2368.

Bigot, K., S. Mignon-Grasteau, M. Picard, and S. Tesseraud. 2003. Effects of delayed feed intake on body, intestine, and muscle development in neonate broilers. *Poult. Sci.* 82:781–788.

Brantsæter, M., F. M. Tahamtani, J. Nordgreen, E. Sandberg, T. B. Hansen, T. B. Rodenburg, R. O. Moe, and A. M. Janczak. 2017. Access to litter during rearing and environmental enrichment during production reduce fearfulness in adult laying hens. *Appl. Anim. Behav. Sci.* 189:49–56.

Bundesamt für Lebensmittelsicherheit und Veterinärwesen (BLV). Fachinformation 16.1: Geflügel, Tauben und Wachteln fachgerecht töten, Fachinformation und Merkblätter, March, 2024, Eidgenössisches Departement des Innern EDI: Bundesamt für Lebensmittelsicherheit und Veterinärwesen. (2024). BLV, Switzerland.

Buschmann, J. U. F., M. Manns, and O. Güntürkün. 2006. Let There be Light!" pigeon eggs are regularly exposed to light during breeding. *Behav. Process.* 73:62–67.

Careghi, C., K. Tona, O. Onagbesan, J. Buyse, E. Decuypere, and V. Bruggeman. 2005. The effects of the spread of hatch and interaction with delayed feed access after hatch on broiler performance until seven days of age. *Poult. Sci.* 84:1314–1320.

Cockrem, J. F. 2007. Stress, corticosterone responses and avian personalities. *J. Ornithol.* 148:S169–S178.

Decuypere, E., K. Tona, V. Bruggeman, and F. Bamerlis. 2001. The day-old chick: A crucial hinge between breeders and broilers. *J. World's Poult. Sci.* 57:135–138.

de Gouw, P., L. J. F. van de Ven, S. Lourens, B. Kemp, and H. van den Brand. 2017. Effects of dust, formaldehyde and delayed feeding on early postnatal development of broiler chickens. *Res. J. Vet. Sci.* 112:201–207.

de Haas, E. N., J. E. Bolhuis, B. Kemp, T. G. G. Groothuis, and T. B. Rodenburg. 2014. Parents and early life environment affect behavioral development of laying hen chickens. *PLoS One* 9:e90577.

de Jong, I. C., A. S. Sander van Voorst, J. H. F. Erkens, D. A. Ehlhardt, and H. J. Blokhuis. 2001. Determination of the circadian rhythm in plasma corticosterone and catecholamine concentrations in growing broiler breeders using intravenous cannulation. *Physiol. Behav.* 74:299–304.

de Jong I. C., J. van Riel, A. Lourens, M. B. M. Bracke, and H. van den Brand. 2016. Effects of food and water deprivation in newly hatched chickens - A systematic literature review and meta-analysis. Wageningen Livestock Research, Report 999. Wageningen, The Netherlands.

de Jong, I. C., J. van Riel, M. B. M. Bracke, and H. van den Brand. 2017. A 'meta-analysis' of effects of post-hatch food and water deprivation on development, performance and welfare of chickens. *PLoS One* 12:e0189350.

de Jong, I. C., H. Gunnink, T. van Hattum, J. W. van Riel, M. M. P. Raaijmakers, E. S. Zoet, and

- H. van den Brand. 2019. Comparison of performance, health and welfare aspects between commercially housed hatchery-hatched and on-farm hatched broiler flocks. *Animal* 13:1269–1277.
- Ericsson, M., and P. Jensen. 2016. Domestication and ontogeny effects on the stress response in young chickens (*Gallus gallus*). *Sci. Rep.* 6:35818.
- Giersberg, M., I. Poolen, K. de Baere, H. Gunnink, T. van Hattum, J. van Riel, and I. de Jong. 2020. Comparative assessment of general behaviour and fear-related responses in hatchery-hatched and on-farm hatched broiler chickens. *Appl. Anim. Behav. Sci.* 232:105100.
- Giersberg, M. F., R. Molenaar, I. C. de Jong, C. Souza da Silva, H. van den Brand, B. Kemp, and T. B. Rodenburg. 2021. Effects of hatching system on the welfare of broiler chickens in early and later life. *Poult. Sci.* 100:100946.
- Gonzales, E., N. Kondo, É. S. P. B. Saldanha, M. M. Loddy, C. Careghi, and E. Decuyper. 2003. Performance and physiological parameters of broiler chickens subjected to fasting on the neonatal period. *Poult. Sci.* 82:1250–1256.
- Gonzales, E., J. H. Stringhini, F. Dahlke, W. C. P. Cunha, and S. A. G. Xavier. 2008. Productive consequences of fasting neonatal chicks of different genetic constitutions for growing. *Braz. J. Poult. Sci.* 10:253–256.
- Hartig, F. 2018. DHARMA: Residual diagnostics for hierarchical (Multi-level/mixed) regression models. (R Package Version 0.1.6.).
- Hedlund, L., R. Whittle, and P. Jensen. 2019. Effects of commercial hatchery processing on short- and long-term stress responses in laying hens. *Sci. Rep.* 9:2367.
- Hemsworth, P. H., G. J. Coleman, J. L. Barnett, and R. B. Jones. 1994. Behavioural responses to humans and the productivity of commercial broiler chickens. *Appl. Anim. Behav. Sci.* 41:101–114.
- Hollemaans, M., S. De Vries, A. Lammers, and C. Clouard. 2018. Effects of early nutrition and transport of 1-day old chickens on production performance and fear response. *Poult. Sci.* 97:2534–2542.
- Holthorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous Inference in General Parametric Models. *Biom. J.* 50:346–363.
- Jessen, C. T., L. Foldager, and A. B. Riber. 2021. Effects of hatching on-farm on behaviour, first week performance, fear level and range use of organic broilers. *Appl. Anim. Behav. Sci.* 238:105319.
- Jenni-Eiermann, S., F. Helfenstein, A. Vallat, G. Glauser, and L. Jenni. 2015. Corticosterone: Effects on feather quality and deposition into feathers. *Methods. Ecol. Evol.* 6:237–246.
- Jones, B. 1993. Reduction of the domestic chick's fear of human beings by regular handling related treatments. *Anim. Behav.* 46:991–998.
- Jones, B., and D. Waddington. 1993. Attenuation of the domestic chick's fear of human beings via regular handling: in search of a sensitive period. *Appl. Anim. Behav. Sci.* 36:185–195.
- Littin, K. E., and J. F. Cockrem. 2001. Individual variation in corticosterone secretion in laying hens. *Br. Poult. Sci.* 42:536–546.
- Lucas, A. M., and P. R. Stettenheim. 1972. Molts and Plumages of Domestic Chickens. Pages 197-233 in *The Avian Anatomy: Integument, Part 1*. US. Agricultural Handbook 362. Agricultural Research Service, Washington, D.C.
- Majer, A. D., R. T. Paitz, G. M. Tricola, J. E. Geduldig, H. P. Litwa, J. L. Farmer, B. R. Prevelige, E. K. McMahon, T. McNeely, Z. R. Sisson, B. J. Frenz, A. D. Ziur, E. J. Clay, B. D. Eames, S. E. McCollum, and M. F. Haussmann. 2023. The response to stressors in adulthood depends on the interaction between prenatal exposure to glucocorticoids and environmental context. *Sci. Rep.* 13:6180.
- Molenaar, R., N. Stockhofe-Zurwieden, M. F. Giersberg, T. B. Rodenburg, B. Kemp, H. van den Brand, and I. C. de Jong. 2023. Effects of hatching system on chick quality, welfare and health of young breeder flock offspring. *Poult. Sci.* 102:102448.
- Monaghan, P., and M. F. Haussmann. 2015. The positive and negative consequences of stressors during early life. *Early Hum. Dev.* 91:643–647.
- Nielsen, B. L., H. R. Juul-Madsen, S. Steinfeldt, J. B. Kjaer, and P. Sørensen. 2010. Environment, well-being, and behavior feeding activity in groups of newly hatched broiler chicks: Effects of strain and hatching time. *Poult. Sci.* 89:1336–1344.
- Olanrewaju, H. A., J. P. Thaxton, W. A. Dozier, J. Purswell, W. B. Roush, and S. L. Branton. 2006. A review of lighting programs for broiler production. *Int. J. Poult. Sci.* 5:301–308.
- Özkan, S., S. Yalçın, E. Babacanoglu, H. Kozanoğlu, F. Karadaş, and S. Uysal. 2012. Photoperiodic lighting (16 hours of light:8 hours of dark) programs during incubation: 1. Effects on growth and circadian physiological traits of embryos and early stress response of broiler chickens. *Poult. Sci.* 91:2912–2921.
- Panda, A. K., S. K. Bhanja, and G. Shyam Sunder. 2015. Early post hatch nutrition on immune system development and function in broiler chickens. *Worlds Poult. Sci. J.* 71:285–296.
- Rogers, L. J. 1995. Environmental Influences on Development of the Embryo. Pages 41-70 in *The Development of Brain and Behaviour in the Chicken*. CABI Publishing, Wallingford, Oxon.
- Rogers, L. J. 2008. Development and function of lateralization in the avian brain. *Brain Res. Bull.* 76:235–244.
- Saito, S., T. Tachibana, Y. H. Choi, D. M. Denbow, and M. Furuse. 2005. ICV melatonin reduces acute stress responses in neonatal chicks. *Behav. Brain. Res.* 165:197–203.
- Scanes, C. G. 2016. Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. *Poult. Sci.* 95:2208–2215.
- Simon, K., G. de Vries Reilingh, B. Kemp, and A. Lammers. 2014. Development of Ileal cytokine and immunoglobulin expression levels in response to early feeding in broilers and layers. *Poult. Sci.* 93:3017–3027.
- Simon, K., G. de Vries Reilingh, J. E. Bolhuis, B. Kemp, and A. Lammers. 2015. Early feeding and early life housing conditions influence the response towards a noninfectious lung challenge in broilers. *Poult. Sci.* 94:2041–2048.
- Sklan, D., Y. Noy, A. Hoyzman, and I. Rozenboim. 2000. Decreasing weight loss in the hatchery by feeding chicks and poults in hatching trays. *J. Appl. Poult. Res.* 9:142–148.
- Souza da Silva, C., R. Molenaar, M. F. Giersberg, T. B. Rodenburg, J. W. van Riel, K. de Baere, I. van Dosselaer, B. Kemp, H. van den Brand, and

- I. C. de Jong. 2021. Day-old chicken quality and performance of broiler chickens from 3 different hatching systems. *Poult. Sci.* 100:100953.
- van den Brand, H., R. Molenaar, I. van der Star, and R. Meijerhof. 2010. Early feeding affects resistance against cold exposure in young broiler chickens. *Poult. Sci.* 89:716–720.
- Van de Ven, L. J. F., A. V. van Wagenberg, P. W. G. Groot Koerkamp, B. Kemp, and H. van den Brand. 2009. Effects of a combined hatching and brooding system on hatchability, chick weight, and mortality in broilers. *Poult. Sci.* 88:2273–2279.
- Wichman, A., R. de Groot, O. Håstad, H. Wall, and D. Rubene. 2021. Influence of different light spectrums on behaviour and welfare in laying hens. *Animals* 11:924.
- Willemsen, H., M. Debonne, Q. Swennen, N. Evaert, C. Careghi, H. Han, V. Bruggeman, K. Tona, and E. Decuyper. 2010. Delay in feed access and spread of hatch: Importance of early nutrition. *Worlds Poult. Sci. J.* 66:177–188.
- Witjes, V. L., R. M. Bruckmaier, S. G. Gebhardt-Henrich, and M. J. Toscano. 2022. Effects of on-farm hatching on short term stress indicators, weight gain, and cognitive ability in layer chicks. *Appl. Anim. Behav. Sci.* 254:105692.
- Yahav, S. 2015. Regulation of Body Temperature: Strategies and Mechanisms. *Strategies and Mechanisms*. Pages 869–905 in *Sturkie's Avian Physiology: Sixth Edition*. Academic Press, Washington, DC ed. C. G. Scanes.
- Yalçın, S., S. Özkan, and T. Shah. 2022. Incubation temperature and lighting: Effect on embryonic development, post-hatch growth, and adaptive response. *Front. Physiol.* 13:899977.
- Zeman, M., P. Pavlik, D. Lamošová, I. Herichová, and E. Gwinner. 2004. Entrainment of rhythmic melatonin production by light and temperature in the chick embryo. *Avian Poult. Biol. Rev.* 15:197–204.
- Zimmer, C., N. J. Boogert, and K. A. Spencer. 2013. Developmental programming: Cumulative effects of increased pre-hatching corticosterone levels and post-hatching unpredictable food availability on physiology and behaviour in adulthood. *Horm. Behav.* 64:494–500.