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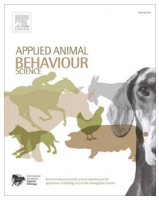

Effects of on-farm hatching on short term stress indicators, weight gain, and cognitive ability in layer chicks

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ABSTRACT

Layer chicks are usually transported early in life, experiencing immediate post-hatch food and water deprivation and various transport-related stressors with potentially negative long-term consequences for learning, cognition and welfare. In contrast, as chicks are only temporarily exposed to these stressors, the experienced stress could be sub-chronic which may improve cognitive flexibility. The aim of this exploratory study was therefore to investigate the acute and long-term effects of on-farm hatching (OFH) compared to conventional hatching. Dekalb White layer chicks were subjected to either OFH (n = 47) with ad libitum access to feed and water or temporary post-hatch resource deprivation and eight hour transport (RDT; n = 42). Physical and behavioural measures were collected to examine short-term effects of the treatment procedures. To determine longer term effects, treatment differences in learning and cognitive flexibility were assessed in a Y-maze using several paradigms (reversal, attentional-shift, extinction) between 4 and 12 weeks of age (WOA). Compared to OFH chicks, RDT had: greater corticosterone levels after transport ($F_{1,19}$ =8.15, p = 0.01, RDT (16.24 ± 1.20 ng/mL) vs. OFH (8.13 ± 1.20 ng/mL) and post-recovery ($F_{1,19}$ =4.93, p = 0.04; RDT (11.69 ± 1.35 ng/mL) vs. OFH (5.31 ± 1.37)), and lower body mass after resource deprivation and transport (F_{2258} =9.7, p < 0.001, RDT (33.14 ± 0.33 g) vs. OFH (37.62 ± 0.28 g)). Performance of activity behaviours (foraging, drinking, resting, wing-assisted running) after transport exhibited treatment by time interactions. Additionally, a tendency for OFH being heavier than RDT chicks was observed up to 11 WOA. The majority of birds learned the initial association in the Y-maze between a reward and location (77% of n = 19 RDT and n = 29 OFH chicks) or light stimulus (91% of n = 12 RDT and n = 11 OFH chicks). Subsequently, a number of chicks reached the learning criterion in the location reversal (24% of n = 13 RDT and n = 24 OFH chicks) and the light-to-location attentional-shift (47% of n = 11 RDT and n = 10 OFH chicks), and most of these chicks succeeded in the following extinction paradigm (80% of n = 3 RDT and n = 7 OFH chicks). No treatment effects were detected in any phase of cognitive testing. In conclusion, treatment affected behaviour and health parameters suggesting RDT animals were recovering from resource deprivation and transport. Continued treatment differences in body mass throughout rearing demonstrated long term effects as well although no effects on initial learning and cognitive flexibility were identified. Future work is needed to determine what mechanisms are responsible for the observed health and behavioural differences.

1. Introduction

Commercially kept layer chicks are typically hatched, sexed, and vaccinated at a hatchery and subsequently transported at one day of age (DOA) to a rearing site. As a consequence, chicks undergo feed and water deprivation until reaching the rearing site, though the ability to consume energy reserves from the yolk sac may attenuate potential negative consequences on their health and welfare (EFSA AHAW Panel, 2011; Freeman, 1982; Malik et al., 2011). Nevertheless, because the hatching window ranges between 24 and 48 h (Careghi et al., 2005; Wang et al., 2020), chicks that hatched earlier may spend substantial time without access to nutritional resources compared to those hatching later. In combination with transportation, these factors may contribute to transport-related mortality (Xin and Lee, 1997).

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Besides resource deprivation, the transportation procedure exposes chicks to an array of stressors (Mitchell and Kettlewell, 2004, 2009a,b). Numerous studies have investigated the effects of transport stress (e.g. shaking, regrouping, noise) on animal health, welfare and behaviour in different species (livestock and poultry: Grandin, 2019; layer chicks, pullets and hens: Mitchell and Kettlewell, 2004, 2009a,b). Since animals are exposed to transport-associated stressors for only hours to days, the experienced stress is suggested to be sub-chronic, decreasing their welfare with only minor or the absence of long-term consequences (National Research Council, 2006). However, since layer chicks are transported at one DOA, this early life stress may have substantial long-lasting effects on subsequent development, behaviour and cognition as demonstrated in various species (humans and rodents: Chen and Baram, 2016; layer chicks and hens: Goerlich et al., 2012; Valros et al., 2008). Severe early life stress has been reported to negatively affect stress responsiveness and learning performance later in life, possibly by disrupting the development of associated brain networks (Chen and Baram, 2016).

Although early life stress can impair initial memory retention, sub-chronic stress can improve cognitive flexibility (Japanese quail: Calandreau et al., 2011; humans and rodents: De Kloet et al., 2005; Joëls et al., 2006), defined as the ability to adapt behaviour to changing environments and reflecting an adaptive capacity (Audet and Lefebvre, 2017). Given the many events and management procedures that may challenge a hen's coping ability, it remains unclear how the magnitude and qualitative effects of early life transport stress will impact subsequent health, cognition and adaptability. For comparison, elimination of immediate post-hatch resource deprivation and transport in broilers through on-farm hatching (OFH) methods resulted in increased body mass and decreased footpad dermatitis prevalence (Jong et al., 2019; Hollemans et al., 2018). Hence, OFH procedures, allowing *in ovo* transport at developmental day 18 and access to resources immediately post-hatch at the rearing site, may have beneficial effects on chick health. In contrast to broilers, only female layer chicks are reared, while males have no function in the production chain (Brümmer et al., 2018; Gremmen et al., 2018). The development of *in ovo* sexing techniques could allow for males to be eliminated in the embryonic stage (Alin et al., 2019; Galli et al., 2018). With the expected incorporation of OFH methods (personal communication, L. van de Ven), these approaches together are likely to make resource deprivation post-hatch, the killing of day-old male chicks, and the transportation of day-old females obsolete.

The aim of this exploratory study was to investigate the short- and long-term effects of OFH procedures, compared to conventional methods that involved resource deprivation and transport (RDT) on laying chick behaviour, health, and cognition. Physical and behavioural measures were collected to examine the effects of treatment on acute stress and health with the expectation that observed differences would have long term consequences. In addition to continued measures of health parameters, several distinct cognitive flexibility paradigms were used to assess the effects of early life resource deprivation and transport on cognition. Our predictions were that RDT chicks would show an increased acute stress response immediately post-transport and after a 20-hour recovery compared to pre-transport values and OFH chicks. We further predicted that a long term difference would exist between OFH and RDT chicks regarding capacity for initial learning and updating associations.

2. Materials and methods

2.1. Ethical statement

All procedures requiring the handling of animals were reviewed and approved by the Veterinary Office of the Canton of Bern, Switzerland (approval number BE101/18). The study was carried out in compliance with the Swiss regulations for experimental animal treatment.

2.2. Animals, housing, and on-farm hatching

For the study, eggs (N = 270; Dekalb-white; mean egg weight: 50.5 g; standard error: ± 0.4 g) from the same parent stock (age: 42 WOA) arrived at the Aviforum facility in Zollikofen, Switzerland, on day 18 of development after an approximate two hour journey. Before transport, non-viable eggs were cleared through candling on developmental day 18. Transportation was done in a commercial vehicle designed for egg transport that maintained an average environmental temperature of 34.6 °C. Eggs were hatched on-farm in four pens (2.5 m high; ground floor litter area: 2.06 ×2.00 m; raised plastic tier: 2.06 ×1.15 m, 0.70 m above the floor) within an environmentally controlled room used for subsequent housing. The walls of the four pens were built from a mesh frame. The raised tiers of the pens were initially divided in half by wooden dividers (115×30×0.5 cm) and an equal number of eggs was allocated to each side of the tiers. Each side of the pen (and the associated eggs) was then assigned either of two treatments: on-farm hatch (OFH; egg weight: 50.1 ± 0.6 g) or resource deprivation and transport (RDT; egg weight: 50.9 ± 0.4 g; n = 33–34/treatment/pen). Eggs were hatched on the raised tier resting in HatchTech Setter Trays (67×57cm; HatchTech B.V. Veenendaal, NL) mounted 20 cm above chick paper and wood shavings. For the OFH treatment, a bell drinker and feeding tray with feed and water, respectively, were provided from placement of the eggs. Following transport of RDT chicks and the 20 h recovery period described below, the wooden dividers were removed resulting in the two treatments being mixed and four replicated pens. Two perches (length*diameter: 2.06 m * 32 mm) were installed at 30 cm and 50 cm above the raised tier in each pen at 3 DOA. At 14 DOA, a ramp on either side of the raised tier was installed and chicks were given access to the litter area below with 1 cm wood shavings.

In order to monitor the hatching procedure, beginning with egg arrival until 24:00 h of day 20 of development, the following measures were taken every three hours: eggshell temperature (Omron Gentle-Temp 521 infra-red ear-thermometer, OMRON Healthcare Europe B.V. Hoofddorp, NL), airspeed at egg and chick level (Testo 405-V1 thermal anemometer, Testo B.V. Almere, NL), and air temperature at egg and tier level (Testo 830-T4 - infra-red thermometer, Testo B.V. Almere, NL). We continually adjusted environmental temperature and humidity to maintain the eggshell temperature between 35 and 38 °C and ambient relative humidity > 30% as the primary objectives. Secondary objectives were to maintain ambient temperature at egg-, chick- and pen-levels above 32 °C, wind speed at egg- and chick-level below 0.15 m/s, and barn CO_2 levels below 2000 ppm. Once it was determined no more eggs would hatch (egg temperature of remaining unhatched eggs began falling), we determined that the overall hatchability was 93% and female hatchability was 48%, within a hatching window duration of ~34 h (see Table S1 for the hatch information). The hatchery normally experiences hatch rates of 35–41%. All animals were sexed by the company managing the post-hatch procedures (Prodavi SA, CH) at one DOA from which 130 females and 123 males were identified. Of all hatched eggs, 89 physically healthy females (using standard commercial criteria) and 72 males were randomly selected and vaccinated according to a standard management schedule (Table S2). The remaining surplus animals were returned to the hatchery and males humanely killed. The male chicks that remained on-site were used for blood collections only (via decapitation and thus killed; described below). The total female chicks housed in the four pens where they were hatched and after males were killed was 21, 22, 23, and 23 from 2 DOA onwards (for number of animals per treatment per pen, see Table S3). The unequal numbers reflected the different hatching rates per pen. At 2 DOA, all female chicks were initially tagged with treatment-specific, colour coded leg rings (Ø5mm) which were replaced with flexible numbered leg rings (Flexiringe Fieger AG Untertuttwil, CH) for individual identification at 5 DOA (Ø5mm), and thereafter replaced at 16 DOA (Ø8mm), and 33 or 39 DOA (Ø12mm).

2.3. Acute stress and long-term health assessments

At 1 DOA (before transport of RDT chicks), the first of three acute stress and health assessments (pre-transport timepoint; Fig. 1) was performed on female chicks by researchers blinded to treatment. All chicks were then assessed immediately after transport (post-transport timepoint) and then again 20 h after transport (i.e. post-recovery timepoint). Only RDT birds were transported though we use the same wording for simplicity. All (female) chicks (OFH: n = 47; RDT: n = 42) were weighed, cloacal temperature measured with the same infra-red ear-thermometer used during hatch, and body condition scored using an adaptation of the PasgarScore© (Boerjan, 2006; Table S4). The animal's ability to right itself and the condition of down, eyes, legs, and beak were scored on a binary scale. At each acute stress and health assessment, 12 males per treatment (i.e, n = 3/pen/-treatment/timepoint) were stunned by cervical dislocation and immediately decapitated to collect blood samples for later corticosterone analysis.

After the pre-transport assessment, female RDT chicks (n = 42) and remaining male RDT chicks (n = 24) were transported within Switzerland for 8 h in an environmentally controlled truck (stocking density: ~16 chicks in a 30 *30 cm tray; average temperature: 27.7 °C), while the OFH chicks (n = 47 females, n = 24 males) remained in their pens with ad libitum access to feed and water. At the conclusion of transport, post-transport stress and health data was collected for all females and RDT chicks were given access to ad libitum feed and water (as with OFH chicks). Following a 20 h recovery period and final post-recovery collection, all chicks were given numbered leg rings and the dividers removed as described above.

During the 20 h recovery period, chicks were video recorded using infra-red-sensitive, video cameras (SNO-L6083RP, Samsung, South Korea) connected to a MULTIEYE network video recorder (artec technologies AG, Diepholz, Germany) to collect behavioural data and later scored by instantaneous sampling at the pen level using specialised software (INTERACT®, Mangold International GmbH, Arnstorf, DE). Every five minutes during a 15 s interval, the number of animals in each pen performing specified activity behaviours (i.e., foraging, drinking, resting and wing-assisted running;) were scored. Number of chicks counted per behaviour were summed during five consecutive hours resulting in four time slots (recovery period: 0–5 h, 5–10 h, 10–15 h and 15–20 h after transport) and expressed relative to number of chicks per treatment-specific side per pen (counts/chick). Intra- and inter-rater reliability scores from 20 samples of two observers, with a minimum of 20 occurrences of each behaviour, ranged from rho= 0.83–0.99 (concordance correlation coefficient).

Data on body mass and body condition parameters (adapted from the PasgarScore©; Boerjan, 2006) were collected weekly between 1 and 12 WOA to assess possible treatment effects on long-term health. Parameters scored were righting ability, eye, leg and beak condition (Table S4). Since no eye infections or injuries and only three instances of minor leg injuries were observed, these parameters were excluded from statistical analysis. All health assessments were conducted by one experimenter (VW) with high intra-observer reliability scores of two repetitions for ability to right itself (from video recordings, n = 46, kappa=0.88, z = 6.02, p < 0.0001), beak condition (n = 46, kappa=0.96, z = 6.49, p < 0.0001) and bodyweight (n = 46, ICC=0.98, F=123, p < 0.0001, 0.97 <95% confidence interval (CI)< 0.99).

2.4. Blood serum collection and corticosterone level analysis

Following blood collection, whole blood was left to clot for 10–30 min at room temperature and stored for 4–9 h at 3 °C. Next,

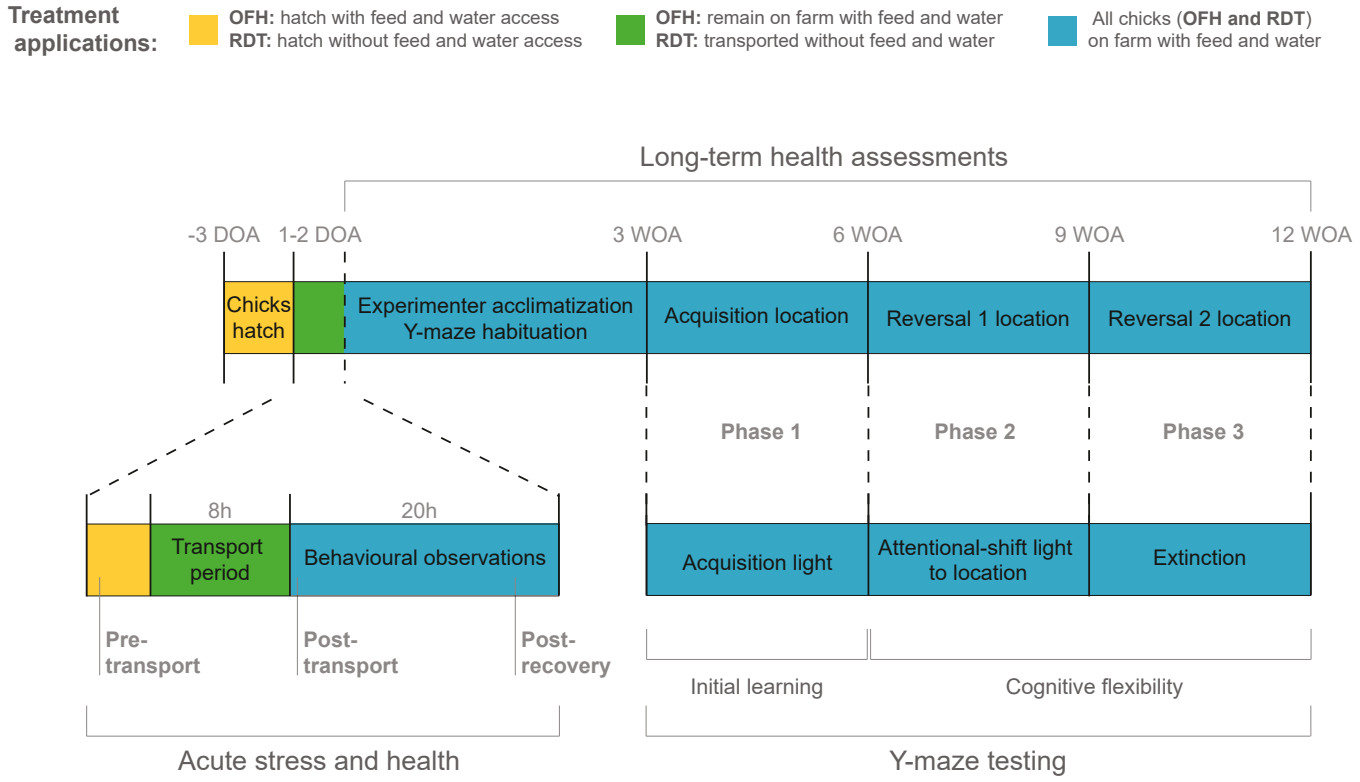

Fig. 1. Experimental timeline. Timeline of experimental procedures (acute stress and health measurements at pre-transport, post-transport and post-recovery timepoints, long-term health assessments from 1 to 12 weeks of age and Y-maze testing during three phases of three weeks per phase) of on-farm hatch (OFH) and resource deprivation and transport (RDT) treatments. During the acute stress and health measurements, treatment differences between OFH and RDT chicks were applied, after which RDT chicks were given access to feed and water and remained on farm. Chicks were allocated to either one of two cognitive paradigms (first and second reversal or attentional-shift and extinction). DOA = days of age; WOA = weeks of age.

samples were centrifuged for 14 min at 2500 rpm, serum collected and stored at − 32 °C. One sample collected at the pre-transport timepoint had to be excluded because of serum clotting. A competitive enzyme immunoassay kit for corticosterone (HS (High Sensitivity)) was used according to the instructions of the producer's manual to measure corticosterone concentrations (analytical sensitivity 0.17 ng/mL, AC-15F1, Immunodiagnostic Systems (IDS) Holdings PLC, Tyne & Wear, UK).

2.5. Cognitive assessment through Y-maze testing

Cognitive abilities were assessed by established tests over several phases (Fig. 2A). Following a period of acclimatisation to the home pen and experimenters and habituation to a Y-maze (see Table S5), initial learning and cognitive flexibility were assessed in relation to treatment. Initial learning speed in associating a conditioning stimulus (i.e. spatial location or light stimulus) to a reward (i.e., a beak size portion of grape) was assessed first. Cognitive flexibility was subsequently evaluated in three distinct paradigms: reversal learning, attentional-shift or extinction, described below in detail.

For the tests, a Y-maze apparatus (Fig. 2B) was custom built and contained a plastic cup (diameter: 7.5 cm, height: 5.5 cm) at the end of each arm to contain a reward. The cups were mounted on a wooden platform (width: 20 cm; height: 2 cm; length: 20 cm) resulting in a double bottom to prevent odour cues. On the back wall of each arm behind the cups, a LED light strip (Arduino nano, ARDUINO®, via Distrelec, Nänikon, CH) was attached which could emit either violet (423 nm; RGB colour codes: 93,0255) or yellow light (598 nm; RGB colour codes: 255,197,0; see Fischer et al., 1975). The colour of the LED-strip in each arm was opposite to the other and randomly changed daily, but was never the same colour for more than two days in a row. The LED lights were activated during all sessions for all testing paradigms. A camera (SNO-L6083RP, Samsung, South Korea) installed above the Y-maze captured all activity during testing sessions.

Between 2 and 7 DOA, animals were acclimatised to the home pen and handling by the researchers who presented grapes by hand. Starting at 8 DOA, chicks began habituation to the Y-maze testing apparatus in gradually decreasing group size for 10 sessions (of 5–10 min per session, depending on group size) over a period of two weeks (see Table S5). During habituation, both cups contained rewards, whereas during testing only one cup was rewarded.

At the start of 4 WOA, birds that showed no or little signs of distress (vocalising, defecating) while in the testing apparatus were selected for testing during three phases of three weeks per phase. To first evaluate initial learning speed (phase 1: acquisition; 4–6 WOA) (Angevaare et al., 2012; Lindqvist et al., 2007), chicks were trained in an association between a conditioned (CS) and unconditioned stimulus (US) using a small piece of grape (Angevaare et al., 2012). For the procedure, the total group of animals were divided into two, mutually exclusive groups where the CS was either spatial location (left or right arm) or light colour (violet or yellow). Within each CS group, both treatments were represented, i.e., CS location (RDT: n = 19; OFH: n = 29) or CS light colour (RDT: n = 12, OFH: n = 11; Fig. 2B). The selected birds were counterbalanced across tests, pens, and stimulus (i.e. right/left, yellow/violet) although use of unequal sample sizes across treatments resulted from a selection error. Pen order for testing chicks was alternated, while chicks within pens were tested in random order.

Chicks were assessed in daily sessions consisting of two consecutive trials (five days a week), with a one-minute inter-trial-interval. For each trial, a bird was placed in the start box and a guillotine door opened

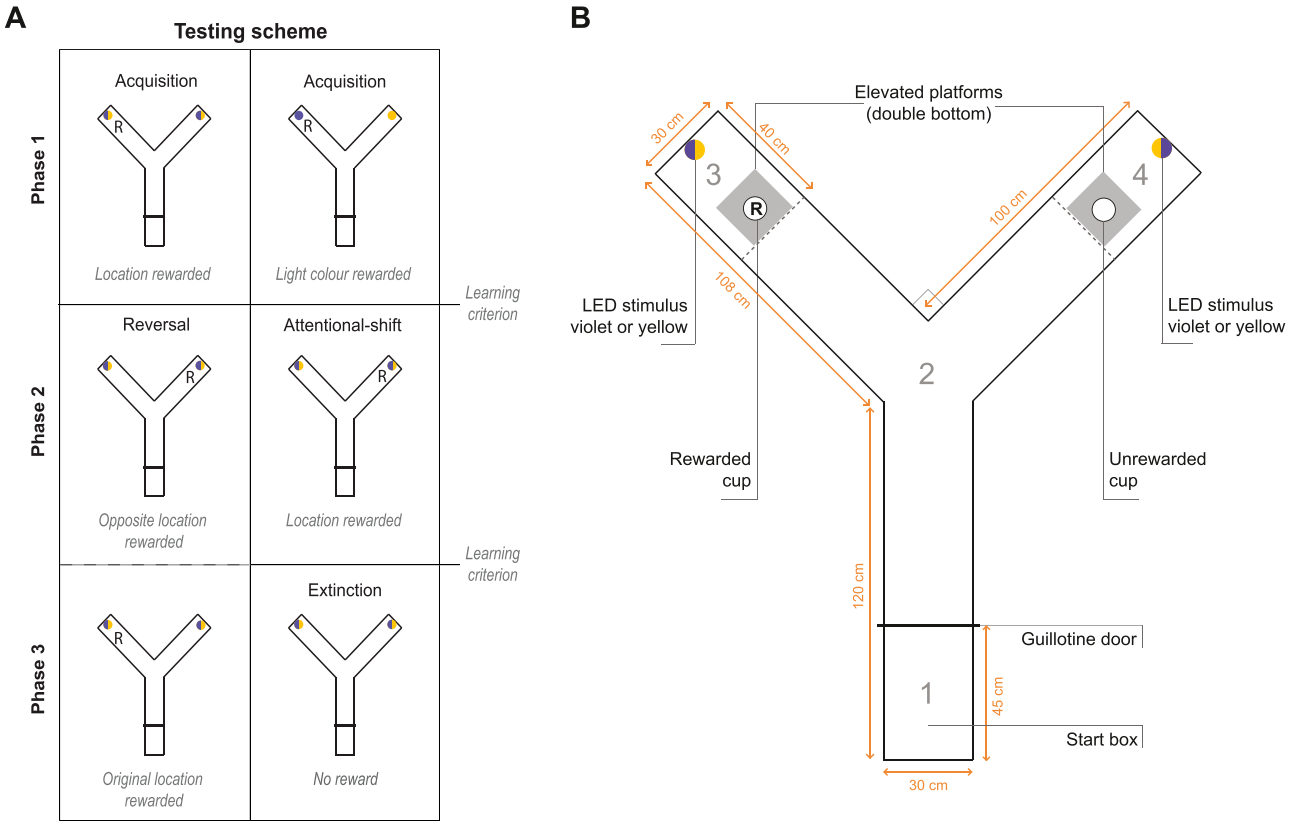

Fig. 2. Y-maze testing schematics and design. (A) Scheme for paradigms performed in Y-maze. During phase 1, a location or light colour was associated with the reward in the reversal- and attentional-shift paradigm, respectively. In phase 2, the location was reversed or the location instead of the light colour became the rewarded stimulus in the reversal- and attentional-shift paradigm, respectively. During phase 3, the rewarded location was reversed to the initial location or no reward was provided in the reversal- and extinction paradigm, respectively. (B) Y-maze design and measurements (see Angevaare et al., 2012). Only one of two cups was rewarded (R) during testing, depending on paradigm, phase and individual chick.

(signifying the beginning of the trial) giving access to the Y-maze corridor and arms after which the experimenter left the room. A trial ended when the chick had entered one of the arms (zone 3 or 4; Fig. 2A) or when three minutes had elapsed. During the first session, chicks were given extra time (max 180 s) after the trial ended with an incorrect choice to explore the other arm of the Y-maze and minimise effects of chance and side/light preferences. When choosing the rewarded side correctly 5/6 times during six consecutive sessions within 15 sessions, the learning criterion was reached and the chick could progress to cognitive testing (phases 2 (7–9 WOA) & 3 (10–12 WOA)). Chicks that failed to meet the learning criterion did not advance to the next phase.

For cognitive flexibility testing (phases 2 & 3), chicks that learned the association with the CS location continued with a reversal paradigm (RDT: n = 13, OFH: n = 24) (Bona et al., 2018) where the opposite location became the CS. Chicks that learned to associate the reward to the CS light colour continued with an attentional-shift paradigm (RDT: n = 11, OFH: n = 10) (adapted from task-switching; Castro and Wasserman, 2016) where the location (instead of light colour) became the CS. Using the same criterion as during the first phase, successful chicks advanced to a third phase where the CS location was reversed to the original reward location (phase 3; RDT: n = 5, OFH: n = 4). Chicks tested in the attentional-shift paradigm entered an extinction paradigm (phase 3; RDT: n = 3, OFH: n = 7) in this phase where no reward was provided (no CS). The extinction test ended when a bird chose the same side of the Y-maze or reached maximum trial duration three out of six times during six consecutive sessions, a milestone interpreted as the chick having 'forgotten' the previously learned association.

The experiment ended when the learning criteria were met or a maximum of 15 sessions were completed. During all phases of the cognitive tasks, the number of trials until reaching criterion was the primary response. For chicks that did not reach the learning criterion within a phase, the minimum number of sessions with correct choices needed to reach the criterion of the current phase was calculated for the analysis (Angevaare et al., 2012). At the end of the experiment, intra-observer reliability scores of two replications were assessed for measuring latency to leave the start box and trial duration from video recordings (n = 20; concordance correlation coefficient, rho>0.999, 0.998 <95%CI<0.999; C_b>0.99 for both parameters) as well as correct/incorrect choice (n = 20, kappa=1, z = 4.47, p < 0.0001).

2.6. Statistical analysis

With the exception of corticosterone concentrations, R statistical software (R Core Team, 2017, version 3.0.2 GUI 1.62) was used with RStudio (R Studio Team, 2020) for all analyses and the limit for statistical significance was set at p < 0.05. Descriptive analyses were carried out following the steps detailed in Zuur et al. (2010). To calculate inter- and intra-observer reliability scores, Cohen's kappa was used (Gamer et al., 2012) for categorical data, intra-class correlation coefficients (ICCs; package 'ICC'; Bartko, 1966) for Gaussian data. Concordance correlation coefficients (CCCs, packages 'epiR' and 'DescTools', Lawrence and Lin, 1989) were used for non-Gaussian data, which are less dependent on assumptions of normality.

Linear Mixed-effect models (LMER; Gaussian data) and generalised linear mixed-effect models (GLMER; non-Gaussian data) were used with packages 'nlme' (Pinheiro et al., 2018) and 'lme4′ (Bates et al., 2015), respectively. As female chicks could only be identified from 5 DOA onwards, repeated measures within pen (but not within individual) could be accounted for in analyses on acute stress and health data. In all other models, the random structure of chick nested in pen was applied. Age or time was either included as a fixed effect or collinear to other fixed effects in the model, depending on the dataset. For Poisson distributed data, over-dispersion was assessed and when found, corrected using negative binomial generalised linear mixed-effects models (package 'MASS', Venables and Ripley, 2002). When residuals were non-random (as visually inspected by Q-Q plots or tested by the package 'DHARMa', (Hartig, 2018)), data was transformed (squared, square rooted, inverted or log transformed; according to the skewedness for Poisson distributed data). When no proper model fit could be achieved, presumably due to low sample sizes, bootstrapping methods were conducted (package "doBy"; Højsgaard, 2012). Non-significant interactions and fixed effects were eliminated from the model by backwards stepwise regression using analyses of variance (ANOVA). When variables were determined to be effective predictors as part of a final model, *post-hoc* analysis was performed (package 'multcomp', Holthorn et al., 2016) for categorical, Gaussian, and Poisson type data with adjusted p-values (Bonferroni method, Shaffer, 1995). For binomial data, the odds ratio (OR) and associated 95% confidence interval were calculated. To confirm learning during the first two phases (phases 1 & 2) of the cognitive paradigms, the performance in the last one or two sessions of the preceding phase was compared to the performance of the first one or two sessions in the next phase by comparing number of trials with correct choices with a paired Wilcoxon-signed rank test. Means are reported as arithmetic means with standard error per treatment.

Corticosterone concentrations were analysed using the Mixed Procedure within SAS (Version 9.4, North Carolina, USA) with treatment as a fixed effect and pen as the subject with repeated measures for each timepoint separately to account for diurnal fluctuations, since time of day differed for the measurements at different timepoints. Residual plots were visually assessed to assure a normal distribution or to determine that a logarithmic transformation was necessary. Means are given as back-transformed least square means in the text where appropriate with treatment specific back-transformed standard errors. Boxplots were produced using raw data.

3. Results

3.1. Acute stress and health assessments

3.1.1. Blood serum corticosterone concentrations

Corticosterone concentration was related to treatment with RDT (16.24 ± 1.20 ng/mL) having increased values compared to OFH (8.13 ± 1.20 ng/mL) at post-transport ($F_{1,19}$ =8.15, p = 0.01) and post-recovery ($F_{1,19}$ =4.93, P = 0.04, 11.69 ± 1.35 ng/mL and 5.31 ± 1.37 ng/mL respectively;). No treatment differences were found at pre-transport ($F_{1,18}$ =2.59, p = 0.13, RDT (6.93 ± 1.26 ng/mL) vs. OFH (11.88 ± 1.27 ng/mL)) (Fig. 3A).

3.1.2. Body mass

Body mass was related to the interaction of treatment and timepoint (F_{2258} =9.7; p < 0.00001). Chicks of the RDT treatment had reduced body mass compared to OFH chicks at pre-transport (p < 0.001, RDT (34.37 ± 0.34 g) vs. OFH (37.41 ± 0.29 g), post-transport (p < 0.001, RDT (33.14 ± 0.33 g) vs. OFH (37.62 ± 0.28 g) and post-recovery (p < 0.01, RDT (38.04 ± 0.34 g) vs. OFH (39.78 ± 0.33 g) (Fig. 3B). Additionally, both OFH and RDT chicks were heavier post-recovery than post-transport (p < 0.001, OFH post-transport vs. OFH post-recovery; p < 0.001, RDT post-transport vs. RDT post-recovery), but RDT did not lose more weight during the transport procedure (p = 0.11, RDT pre-transport vs. RDT post-transport).

3.1.3. Cloaca temperature

Cloaca temperature was related to an interaction of treatment and timepoint (F_{2258} =5.7; p < 0.01). Pre-transport, RDT (38.75 ± 0.07 °C) had reduced temperatures compared to OFH (39.14 ± 0.08 °C) chicks (p < 0.001), while post-transport and post-recovery, no difference was detected (p > 0.1) (Fig. 3C). Within treatments, both OFH chicks and RDT chicks had higher temperatures post-transport than pre-transport (p < 0.001, OFH - pre-transport vs. post-transport (39.63 ± 0.05 °C); p < 0.001, RDT - pre-transport vs. post-transport (39.56 ± 0.07 °C)) and lower temperatures post-recovery than post-transport (p < 0.001, OFH - post-transport vs. post-recovery (39.03 ± 0.08 °C); p < 0.001, RDT -

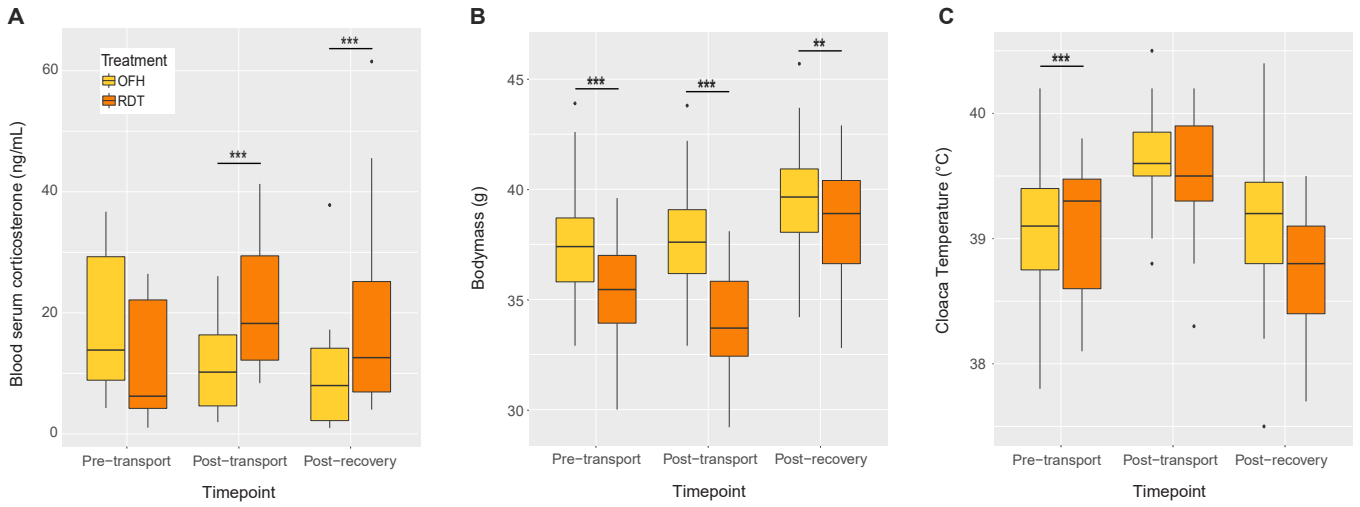

Fig. 3. Serum corticosterone, body mass and cloaca temperature over treatments and timepoints. (A) Blood serum corticosterone concentrations (ng/mL) of male on-farm hatch (OFH, yellow, n = 12/timepoint) and resource deprivation and transport (RDT, orange, n = 12/timepoint) treatments, (B) Body mass (g) and (C) Cloaca temperature (°C) of female on-farm hatch (OFH, yellow, n = 47) and resource deprivation and transport (RDT, orange, n = 42) treatments at pre-transport, post-transport and post-recovery timepoints. Boxplot horizontal lines indicate medians, boxes indicate interquartile range and vertical lines indicate 1.5 times the interquartile range from the top or bottom of the box to the furthest data point within that distance. Any data beyond that are represented as individual points. **P < 0.01, ***P < 0.001.

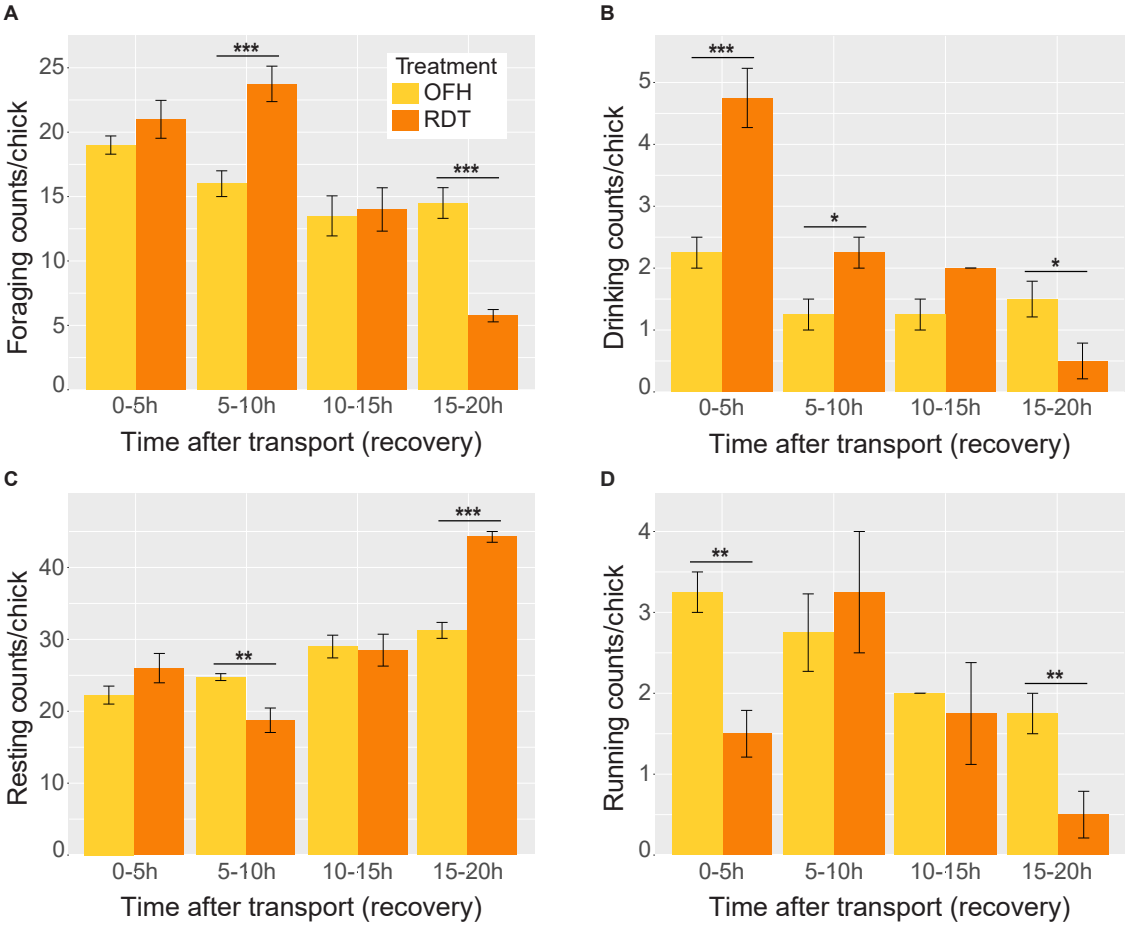

Fig. 4. Behavioural counts during recovery period. Mean number of counts for on-farm hatch (OFH; yellow bars, n = 4) and resource deprivation and transport (RDT; orange bars, n = 4) chicks during 0–5 h, 5–10 h, 10–15 h and 15–20 h time slots after transport are displayed for (A) foraging counts; (B) drinking counts; (C) resting counts; and (D) wing-assisted running counts. Vertical lines indicate standard errors. *P < 0.05, **P < 0.01, ***P < 0.001.

post-transport vs. post-recovery (39.07 ± 0.07 °C)).

3.1.4. Righting ability

Ability to right oneself was related to treatment (F_{1258} =8.4, p = 0.003) and timepoint (F_{2258} =2.6, p = 0.04). Greater OR of poor ability were found for RDT (38% of chicks) compared to OFH chicks (21% of chicks) (OR=2.31, 1.47 <95%CI<3.66; Table S6), Odds for poor ability were greater post-transport (33% of chicks) than pre-transport (19% of chicks) (OR=2.28, 1.33 <95%CI<3.95) and post-recovery (33% of chicks) than pre-transport (OR=2.02, 1.14 <95% CI<3.60).

3.1.5. Behavioural counts during recovery period

The number of foraging counts was related to an interaction of time slot and treatment ($F_{3,21}$ =34.54; p < 0.001). More foraging counts were observed during the period 5–10 h after transport in RDT (23.75 ± 1.38 counts/chick) than OFH chicks (16.00 ± 1.00 counts/chick) (p < 0.001; Fig. 4A). The opposite pattern was seen 15–20 h after transport where OFH (14.50 ± 1.19 counts/chick) showed more foraging counts than RDT chicks (5.75 ± 0.48 counts/chick) (p < 0.001; see Table S7 for all relevant within/between treatments comparisons).

For drinking behaviour, treatment by time interactions ($F_{3,21}$ =13.36; p < 0.001) were identified. More drinking counts were detected for RDT (4.75 ± 0.48 counts/chick) in comparison to OFH chicks (2.25 ± 0.25 counts/chick) during 0–5 h after transport (p < 0.001; Fig. 4B) with the same pattern at 5–10 h (2.25 ± 0.25 counts/chick and 1.25 ± 0.25 counts/chick respectively) (p < 0.05). In the period during 15–20 h after transport, OFH (1.50 ± 0.29 counts/chick) showed more drinking counts than RDT chicks (0.50 ± 0.29 counts/chick) (p < 0.05).

The number of resting counts related to the interaction of treatment and time ($F_{3,21}$ =14.43; p = 0.0001). Less resting counts were observed for RDT (18.75 ± 1.70 counts/chick) 5–10 h after transport than OFH chicks (24.75 ± 0.48 counts/chick) (p < 0.01; Fig. 4C), while the opposite was seen 15–20 h after transport (44.25 ± 0.75 counts/chick and 31.25 ± 1.11 counts/chick respectively) (p < 0.001).

Treatment and time interacted ($F_{3,21}$ =6.84; p < 0.01) in relation to wing-assisted running counts. During 0–5 h after transport, less running counts were detected for RDT (1.50 ± 0.29 counts/chick) than for OFH chicks (3.25 ± 0.25 counts/chick) (p < 0.01; Fig. 4D) with a similar pattern at 15–20 h (0.50 ± 0.29 counts/chick and 1.75 ± 0.25 counts/chick respectively) (p < 0.01).

3.2. Long-term health assessments

3.2.1. Body mass

Body mass was found to increase according to a polynomial function across DOA and therefore, linear, squared and cubed functions of DOA were included in the final model. A function of treatment ($F_{1,83}$ =4.00, p < 0.05), cubed DOA (F_{1964} =878.1, p < 0.001) and the interaction of treatment by DOA (F_{1964} =5.70, p < 0.05) were identified. Plotting the model estimates with 95% confidence intervals showed a pattern for OFH chicks to be heavier than RDT chicks, although differences were inconsistent and equalised by the end of the experiment (Fig. 5).

3.2.2. Righting ability and beak condition

There was no interaction between treatment and DOA in relation to righting ability so only main factors were included in the final model. There was no main effect of treatment, while a possible effect of DOA was detected (F_{1964} =3.71, p < 0.01), but the odds ratio indicated no relationship (OR=1.01, 1.00 <95%CI<1.01).

Beak condition was related to DOA (F_{1964} =144.13, p < 0.01) with the odds for a poor beak condition increasing with DOA (OR=1.09, 1.07 <95%CI<1.11), although no relationship with treatment or DOA interaction was found.

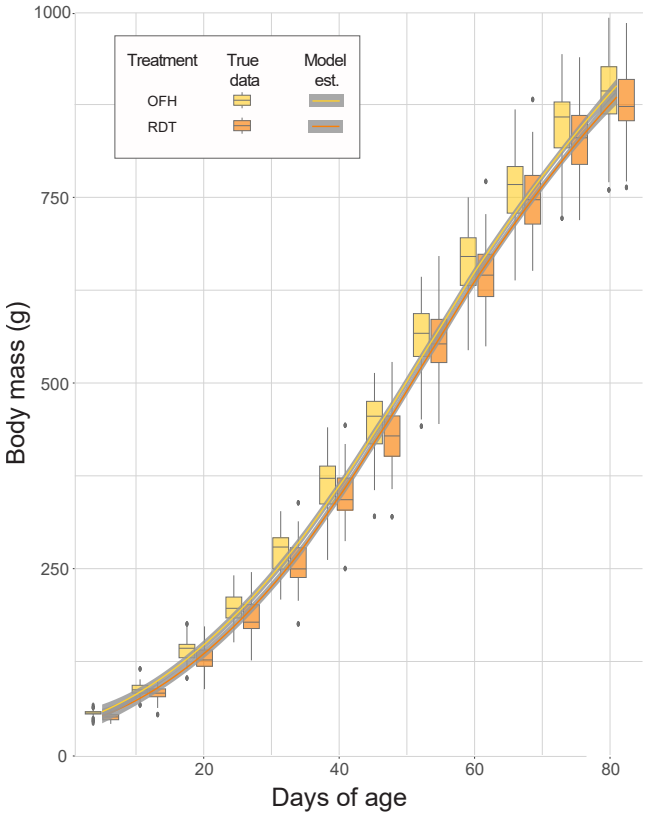

Fig. 5. Model estimates and boxplots of body mass across days of age. Body mass (gram) model estimates (est.) with 95% confidence intervals of on-farm hatch (OFH; yellow, n = 47) and resource deprivation and transport (RDT; orange, n = 41) treatments across days of age plotted over actual body mass (gram) for both treatments. Horizontal lines within boxplots indicate medians, boxes indicate interquartile range and vertical lines indicate 1.5 times the interquartile range from the top or bottom of the box to the furthest data point within that distance. Results beyond that range are represented as individual points.

3.3. Initial learning and cognitive flexibility in Y-maze paradigms

3.3.1. Reversal paradigm

Mean trial duration (OFH: 4.90 ± 0.21 s; RDT: 5.58 ± 0.40 s) and latency to leave the start box (OFH: 0.52 ± 0.02 s; RDT: 0.79 ± 0.17 s) during the first phase (phase 1, acquisition) of the reversal paradigm was well below the maximum trial duration of 180 s, indicating the chicks were habituated to the Y-maze task. Comparisons of learning speed when associating the reward to the left or right side found that rewarded side was not related to the number of trials needed to reach criterion (phase 1: $F_{1,46}$ = 1.17, p = 0.28, right (n = 24; 10.38 ± 1.02 trials) vs. left (n = 24; 12.00 ± 1.15 trials)). A covariate of choosing the correct side due to chance or side preference during the first session was initially included in the model, but ultimately was not an effective predictor. Treatment did not relate to learning speed of the initial association (phase 1: $F_{1,46}$ =0.35, p = 0.55, OFH (n = 29; 10.76 ± 1.00 sessions) vs. RDT (n = 19; 11.84 ± 1.22 sessions)).

Chicks that reached the learning criterion (77% of n = 48 chicks) had more correct trials at the end of phase 1 (phase 1: n = 37 animals; 3.76 ± 0.07 out of four trials correct) than at the start of phase 2 (phase 2: 0.51 ± 0.13 out of four trials correct) which confirmed learning in the first phase (paired Wilcoxon signed rank test, p < 0.0001). No effect of the number of sessions until reaching the learning criterion in phase 1 nor its interaction with treatment was found on the number of sessions until reaching criterion in phase 2. Lastly, treatment had no relationship on the number of sessions required to reach the learning criterion in the

first reversal (phase 2: $F_{1,35}$ =0.23, p = 0.63, OFH (n = 24; 17.58 ± 0.55 sessions) vs. RDT (n = 13; 17.00 ± 0.69 sessions)).

Comparison of number of correct choices of chicks again reaching the learning criterion (24% of n = 37 chicks) similarly confirmed learning in phase 2 (paired Wilcoxon signed rank test, p < 0.0001, n = 9 animals, end phase 2: 4.00 ± 0.00 out of four trials correct, start phase 3: 0.67 ± 0.24 out of four trials correct). Since very few chicks (n = 9) reached the third phase (phase 3, second reversal) and only two reached the learning criterion in this phase (22% of n = 9 chicks), only the effect of treatment was tested which was found to be unrelated to the number of trials to learn the second reversal (phase 3: $F_{1,7}$ =0.16; p = 0.70, OFH (n = 4; 17.75 ± 0.48 sessions) vs. RDT (n = 5; 15.80 ± 1.46 sessions)). Additional correlations to the number of sessions needed to attain the learning criterion in earlier phases were inspected visually and showed no discernible pattern.

3.3.2. *Attentional-shift and extinction paradigm*

For the acquisition (phase 1), mean trial duration (OFH: 5.31 ± 0.55 s; RDT: 4.74 ± 0.39 s) and latency to leave the start box (OFH: 0.67 ± 0.09 s; RDT: 0.81 ± 0.24 s) was low, implying chicks were properly habituated. An effect of light colour on learning speed was detected ($F_{1,21}$ =11.41, p < 0.0001) and chicks learned the association faster when the reward was associated to the violet light compared to the yellow light (phase 1: p < 0.0001, violet (n = 11; 7.00 ± 0.52 trials) vs. yellow (n = 12; 11.33 ± 0.93 trials)). However, this did not affect overall learning speed across treatments, since no interaction effect of treatment by light colour or treatment effects were found. Choosing the correct light colour during the first session of the acquisition had no effect on later responses, and treatment did not relate to the number of trials until reaching criterion (phase 1: $F_{1,21}$ =0.01; p = 0.91, OFH (n = 11; 9.18 ± 1.14 sessions) vs. RDT (n = 12; 9.33 ± 0.91 sessions)).

The number of correct choices during the last session of phase 1 versus the first session of phase 2 indicated chicks reaching the learning criterion (91% of n = 23 chicks) had learned the initial association (paired Wilcoxon signed rank test, p < 0.0001, n = 21 animals, end phase 1: 1.90 ± 0.07 out of two trials correct, start phase 2: 0.05 ± 0.05 out of two trials correct). Both the number of sessions needed to reach criterion in the second phase (phase 2, attentional-shift) and its interaction with treatment did not relate to the number of sessions required to reach the learning criterion in phase 2. A tendency for OFH chicks to have a lower number of sessions until reaching criterion compared to RDT chicks in the attentional-shift was found (phase 2: $F_{1,19}$ =2.79, p = 0.08, OFH (n = 10; 15.00 ± 1.04 sessions) vs. RDT (n = 11; 17.45 ± 0.80 sessions)).

For chicks that reached the second learning criterion (47% of n = 21 chicks), a comparison of the number of correct choices during the last session of phase 2 versus the first session of phase 3, where an incorrect choice was counted when the chick chose the previously rewarded side, indicated learning in phase 2 (paired Wilcoxon signed rank test, p < 0.01, n = 10 animals, end phase 2: 1.80 ± 0.13 out of two trials correct, start phase three: 0.30 ± 0.15 out of two trials correct). Only a few birds (n = 10) attained the learning criterion to reach the third phase (phase 3, extinction) and of these birds, 80% reached the criterion for extinction. Therefore, only the effect of treatment was tested. Possible correlations to number of sessions needed to reach criterion in earlier phases were visually inspected, however no obvious correlations were detected. Treatment did not relate to the number of sessions to reach the learning criterion (phase 3: $F_{1,8}$ =0.00; p = 0.97, OFH (n = 7; 10.43 ± 1.31 sessions) vs. RDT (n = 3; 10.33 ± 2.96 sessions)).

4. Discussion

The current study sought to examine implications for on-farm hatching (OFH) and related procedures that are expected to be implemented with hatching of female-only laying hens. In this likely scenario, chicks would be hatched on site (rather than in a hatchery as is standard now) in a manner comparable to that commonly done with broilers where both males and females are raised. Broilers OFH routines generally have positive outcomes on health, though long term implications are unknown. Additionally, given the very different genetic selection and life processes of laying hens, research on the topic is required.

The RDT treatment procedures increased activation of the hypothalamic-pituitary-axis as observed with greater blood corticosterone concentrations, altered behavioural patterns and reduced body mass. Our results are comparable to the findings of others who demonstrated that processes typical of commercial hatching for layers and broilers in the 24-hour window following hatch can have short-term effects including increased corticosterone availability (Hedlund et al., 2019), physical changes (De Jong et al., 2020; Hollemans et al., 2018), and behavioural responses (Giersberg et al., 2020b; Hedlund et al., 2019). Exposure to varying treatments in the immediate post-hatch period seem to have long-term consequences extending beyond the first week of life in terms of body mass as seen in the current study and by others (De Jong et al., 2020; Hedlund et al., 2019; Hedlund and Jensen, 2021). Other reported long-term effects include hatchery-hatched chicks exhibiting increased footpad dermatitis (Giersberg et al., 2021) and less fearfulness during a novel arena test (Giersberg et al., 2020b). Thus, our findings add to a growing body of evidence that chicks are affected by procedures during this critical period, although the specific processes and the duration of the effects remain unclear. For instance, by comparing with their previous findings (Hedlund et al., 2019), Hedlund and Jensen (2021) suggested that hatching in the commercial hatchery by itself was a major source of stress which was worsened by the additional standard, post-hatch processing (e.g., sexing, vaccination, counting, and sorting). Giersberg et al. (2020a) focused on conveyance characteristics (speed, acceleration, drop height), but saw no treatment differences in terms of long-term behavioural or welfare indicators.

We believe our study contributes to the understanding of events in the post-hatch period and demonstrates that the differences observed in the current study are likely independent of the physical hatch environment and processing, but instead depend on transportation and access to feed and water. Regarding causative processes, our study involved two procedures that are likely to contribute to observed differences and should be isolated for comparison in future studies – 1) immediate access to feed and water (versus restriction) following hatch and 2) absence or presence of early life transport. Chicks of each treatment were hatched adjacent with the OFH treatment being observed to readily feed within several hours of dropping from the hatch crate. Although it was not possible to quantify feeding, differences in body mass reported here and elsewhere, including specific organ weights (De Jong et al., 2020), suggested birds were feeding and deriving benefits. Occurrence of feeding is further supported by the higher cloacal temperature observed in OFH chicks pre-transport, which could be explained by the difference in resource access between the treatments (Hollemans et al., 2018; van den Brand et al., 2010), although the difference was not detected at the post-transport timepoint immediately following the period where RDT chicks remained without feed and water. Giersberg et al. (2021) did not observe large differences in broiler welfare or behaviour (i.e., only footpad dermatitis) when comparing commercial hatchers with and without access to food and water, though the use of different protocols limit interpretation. After all chicks were hatched in the current study, RDT chicks were transported for an eight-hour transport that represents the maximum transport time allowed in Switzerland, which is a comparatively brief compared to many parts of the world where 30 h of transport by plane and/or truck are typical (Mitchell and Kettlewell, 2004). The finding of reduced righting ability following transportation and during the period of recovery indicates that animals were likely exhausted. Transportation is well known to be stressful for poultry (Knowles, 1994; Mitchell and Kettlewell, 2009a; Valros et al., 2008), with the loading and unloading of animals known to be particularly impactful (Mitchell and Kettlewell,

2009b). Although it is not possible to parse the individual effects of access to feed and water versus early life transport, we do believe the side-by-side hatching within this study indicates that these factors are both impactful.

Despite the long term effect of differences in body mass reported here and of behavioural differences reported by others, we were unable to detect any widespread treatment effect in cognitive ability. Several explanations for the lack of an effect are possible. Firstly, the relatively low number to reach the learning criterion in all treatments may indicate the tests were not well suited to the capacities of the chicks. A large proportion of birds fared well in the initial association (phase 1) for both paradigms, but success fell dramatically in the first test for cognitive flexibility (i.e., phase 2) leading to low sample sizes in the last phase (phase 3). Chicks have a relatively fixed responsiveness to key maternal cues at this early stage of life (Versace et al., 2017) and have evolved to learn specific associations from the mother hen whom herself is known to vary her behaviour so that chicks will perform the task correctly (Nicol and Pope, 1996). Consequently it may be advantageous for chicks to be relatively inflexible in their associative abilities, especially early in life while still dependent on the mother hen. Nevertheless, cognitive paradigms with comparable difficulty have been successfully completed by laying chicks of similar ages (Nordquist et al., 2011). Furthermore, although chicks were able to acquire the basic associations, the additional light/location stimulus present in all phases of this study may have overwhelmed the capacities of the animals, especially in line with our earlier suggestion of inflexible associations.

Secondly, poultry are fairly resilient to a variety of stressful conditions and the resource deprivation and transport stressors applied in this study may not have been severe enough to induce cognitive effects, despite being experienced during early life. For comparison, adult laying hens are generally productive (e.g. egg production) in diverse housing environments (e.g. cage, aviary, free-range access, etc.) and management strategies (Karcher et al., 2015; Shimmura et al., 2010), despite various behavioural abnormalities (e.g., feather pecking (Cronin and Glatz, 2020; Fijn et al., 2020) and piling (Winter et al., 2021)) that are likely impacted by early life experiences (Cronin and Glatz, 2020; Rodenburg et al., 2004). Clearly, production endpoints are only one type of measure and animal welfare should include a broad array of indicators including the potential for suffering (Broom, 1991; Dawkins, 2008). Although no long-term cognitive effects of transport and resource deprivation at one day of age were identified here, early life stress may differentially affect abnormal behaviours that appear later in life, and further research is warranted to assess the potential long-term consequences of OFH compared to RDT.

Given the absence of adverse effects found for OFH, it is important to consider the likely benefits, including towards welfare and production. With direct access to feed and water post-hatch and the elimination of transport at one DOA, mortality during and after transport as a consequence of dehydration and malnutrition could be minimal or even absent (Weeks, 2014; Xin and Lee, 1997). Furthermore, OFH is generally easier from a management perspective than conventional hatching as, assuming the room temperature is kept below a critical threshold, generally higher hatch rates are found such as those in the current study (compared to the same batch that remained in the hatchery and was independent of our effort), in follow up studies by our group (*Unpublished results*), and reported elsewhere in broilers (de Jong et al., 2019). Dramatic benefits in hatch rates seen with broilers have led several companies to convert entirely to on-farm hatch operations (Personal communication, S. Vonk). Assuming sexing will no longer be required and needed procedures for laying hens will be developed (e.g. vaccinations that can be administered to groups instead of individuals), OFH offers an exciting opportunity to reduce stress during the hatching period while yielding impressive production gains.

5. Conclusion

Hatching of chicks on-farm is likely to become a standard practice with the advent of female only hatching processes. Although clear short-term stress and limited long term responses were observed suggesting the condition of on-farm hatch was less stressful, no convincing differences in cognitive abilities were observed. We believe future work is needed to determine specific mechanisms that are responsible for the observed health and behavioural differences.

CRediT authorship contribution statement

Vivian L. Witjes: conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article. **Rupert M. Bruckmaier:** acquisition of data. **Sabine G. Gebhardt-Henrich:** conception and design of the study, analysis and interpretation of data, revising article critically for important intellectual content. **Michael J. Toscano:** conception and design of the study, interpretation of data, drafting the article, revising article critically for important intellectual content.

Declaration of Competing Interest

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.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2022.105692.

References

Alin, K., Fujitani, S., Kashimori, A., Suzuki, T., Ogawa, Y., Kondo, N., 2019. Non-invasive broiler chick embryo sexing based on opacity value of incubated eggs. Comput. Electron. Agric. 158, 30–35.

Angevaare, M.J., Prins, S., van der Staay, F.J., Nordquist, R.E., 2012. The effect of maternal care and infrared beak trimming on development, performance and behavior of Silver Nick hens. Appl. Anim. Behav. Sci. 140 (1–2), 70–84. https://doi.org/10.1016/j.applanim.2012.05.004.

Audet, J.-N., Lefebvre, L., 2017. What's flexible in behavioral flexibility? Behav. Ecol. 28 (4), 943–947. https://doi.org/10.1093/beheco/arx007.

Bartko, J.J., 1966. The intraclass correlation coefficient as a measure of reliabilit. Psychol. Rep. 19, 3–11.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting LInear Mixed-effects Models Using {lme4}. J. Stat. Softw. 67 (1), 1–48. https://doi.org/10.18637/jss.v067.i01.

Boerjan, M., 2006. Chick vitality and uniformity. Int. Hatch. Pract. 20 (8), 8–9.

Bona, L., Van Staaveren, N., Pokharel, B.B., van Krimpen, M., Harlander-Matauschek, A., 2018. The effect of low protein energy-rich diets on plasma hepatic markers, hepatic damage, and discrimination reversal learning in young female chicks. Front. Vet. Sci. 5, 107.

van den Brand, H., Molenaar, R., van der Star, I., Meijerhof, R., 2010. Early feeding affects resistance against cold exposure in young broiler chickens. Poult. Sci. 89 (4), 716–720.

Broom, D.M., 1991. Animal welfare: concepts and measurements. J. Anim. Sci. 69 (10), 4167–4175.

Brümmer, N., Christoph-Schulz, I., Rovers, A., 2018. Consumers' perspective on dual-purpose chickens as alternative to the killing of day-old chicks. Int. J. Food Syst. Dyn. 9 (5), 390–398.

Calandreau, L., Bertin, A., Boissy, A., Arnould, C., Constantin, P., Desmedt, A., Guémené, D., Nowak, R., Leterrier, C., 2011. Effect of one week of stress on emotional reactivity and learning and memory performances in Japanese quail. Behav. Brain Res. 217 (1), 104–110. https://doi.org/10.1016/j.bbr.2010.10.004.

Careghi, C., Tona, K., Onagbesan, O., Buyse, J., Decuypere, E., Bruggeman, V., 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 (8), 1314–1320. https://doi.org/10.1093/ps/84.8.1314.

Castro, L., Wasserman, E.A., 2016. Executive control and task switching in pigeons. Cognition 146, 121–135. https://doi.org/10.1016/j.cognition.2015.07.014.

Chen, Y., Baram, T.Z., 2016. Toward understanding how early-life stress reprograms cognitive and emotional brain networks. Neuropsychopharmacology 41 (1), 197–206. https://doi.org/10.1038/npp.2015.181.

Cronin, G.M., Glatz, P.C., 2020. Causes of feather pecking and subsequent welfare issues for the laying hen: a review. Anim. Prod. Sci.

Dawkins, M.S., 2008. The science of animal suffering. Ethology 114 (10), 937–945.

De Jong, I.C., Van Hattum, T., Van Riel, J.W., De Baere, K., Kempen, I., Cardinaels, S., Gunnink, H., 2020. Effects of on-farm and traditional hatching on welfare, health, and performance of broiler chickens. Poult. Sci. 99 (10), 4662–4671. https://doi.org/10.1016/j.psj.2020.06.052.

De Kloet, E.R., Joëls, M., Holsboer, F., 2005. Stress and the brain: from adaptation to disease. Nat. Rev. Neurosci. 6 (6), 463–475. https://doi.org/10.1038/nrn1683.

EFSA AHAW Panel, 2011. European Food Safety Authority: scientific opinion concerning the welfare of animals during transport. EFSA J. 9 (1), 1966. https://doi.org/10.2903/j.efsa.2011.1966.

Fijn, L.B., Staay, F., Goerlich-Jansson, V.C., Arndt, S.S., 2020. Importance of basic research on the causes of feather pecking in relation to welfare. Animals 10 (2), 213.

Fischer, G.J., Morris, G.L., Ruhsam, J.P., 1975. Color pecking preferences in white leghorn chickens: erratum. J. Comp. Physiol. Psychol. 89 (7), 802. https://doi.org/10.1037/h0078149.

Freeman, B.M., 1982. Transportation of poultry. World's Poult. Sci. J. 40 (1), 19–30.

Galli, R., Preusse, G., Schnabel, C., Bartels, T., Cramer, K., Krautwald-Junghanns, M.-E., Koch, E., Steiner, G., 2018. Sexing of chicken eggs by fluorescence and Raman spectroscopy through the shell membrane. PLoS One 13 (2), e0192554.

Gamer, M., Lemon, J., Gamer, M.M., Robinson, A., Kendall, W., 2012. Package "irr". Various Coefficients of Interrater Reliability and Agreement, p. 22.

Giersberg, M.F., Molenaar, R., Pieters, R., Boyer, W., Rodenburg, T.B., 2020a. Effects of drop height, conveyor belt speed, and acceleration on the welfare of broiler chickens in early and later life. Poult. Sci. 99 (12), 6293–6299.

Giersberg, M.F., Poolen, I., de Baere, K., Gunnink, H., van Hattum, T., van Riel, J.W., de Jong, I.C., 2020b. 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., Molenaar, R., de Jong, I.C., da Silva, C.S., van den Brand, H., Kemp, B., Rodenburg, T.B., 2021. Effects of hatching system on the welfare of broiler chickens in early and later life. Poult. Sci. 100 (3), 100946.

Goerlich, V.C., Nätt, D., Elfwing, M., Macdonald, B., Jensen, P., 2012. Transgenerational effects of early experience on behavioral, hormonal and gene expression responses to acute stress in the precocial chicken. Horm. Behav. 61 (5), 711–718. https://doi.org/10.1016/j.yhbeh.2012.03.006.

Grandin, T., 2019. Livestock Handling and Transport. CABI.

Gremmen, B., Bruijnis, M.R.N., Blok, V., Stassen, E.N., 2018. A public survey on handling male chicks in the Dutch egg sector. J. Agric. Environ. Ethics 31 (1), 93–107.

Hartig, F., 2018. DHARMa: Residual Diagnostics for Hierarchical (Multi-level/mixed) Regression Models. (R Package Version 0.1.6.).

Hedlund, L., Jensen, P., 2021. Incubation and hatching conditions of laying hen chicks explain a large part of the stress effects from commercial large-scale hatcheries. Poult. Sci. 100 (1), 1–8. https://doi.org/10.1016/j.psj.2020.10.015.

Hedlund, L., Whittle, R., Jensen, P., 2019. Effects of commercial hatchery processing on short-and long-term stress responses in laying hens. Sci. Rep. 9 (1), 1–10.

Højsgaard, S., 2012. The doBy Package (R Package Version 4(3)).

Hollemans, M.S., de Vries, S., Lammers, A., Clouard, C., 2018. Effects of early nutrition and transport of 1-day-old chickens on production performance and fear response. Poult. Sci. 97 (7), 2534–2542. https://doi.org/10.3382/ps/pey106.

Holthorn, T., Bretz, F., Westfall, P., Helilberger, R.M., Schuetzenmeister, A., Scheilbe, S., Hothorn, M.T., 2016. Package 'multcomp'. Simultaneous Inference in General Parametric Models. Project for Statistical Computing.

Joëls, M., Pu, Z., Wiegert, O., Oitzl, M.S., Krugers, H.J., 2006. Learning under stress: how does it work? Trends Cogn. Sci. 10 (4), 152–158. https://doi.org/10.1016/j.tics.2006.02.002.

de Jong, I.C., Gunnink, H., van Hattum, T., van Riel, J.W., Raaijmakers, M.M.P., Zoet, E. S., van den Brand, H., 2019. Comparison of performance, health and welfare aspects between commercially housed hatchery-hatched and on-farm hatched broiler flocks. Animal 13 (6), 1269–1277. https://doi.org/10.1017/S1751731118002872.

Karcher, D.M., Jones, D.R., Abdo, Z., Zhao, Y., Shepherd, T.A., Xin, H., Al, K.E.T., 2015. Impact of commercial housing systems and nutrient and energy intake on laying hen performance and egg quality parameters 1. Poult. Sci. 94, 485–501.

Knowles, T.G., 1994. Handling and transport of spent hens. World Poult. Sci. J. 50 (1), 60–61.

Lawrence, I., Lin, K., 1989. A concordance correlation coefficient to evaluate reproducibility. Bometrics 45, 255–268.

Lindqvist, C., Janczak, A.M., Nätt, D., Baranowska, I., Lindqvist, N., Wichman, A., Lundeberg, J., Lindberg, J., Torjesen, P.A., Jensen, P., 2007. Transmission of stress-induced learning impairment and associated brain gene expression from parents to offspring in chickens. PLoS ONE 2 (4). https://doi.org/10.1371/journal.pone.0000364.

Malik, H.E.E., Ali, O.H.A., Elhadi, H.M., Elzubeir, E.A., 2011. Residual yolk utilization in fast and slow-growing chicks, subjected to feed and water deprivation. Asian J. Biol. Sci. 4 (1), 90–95. https://doi.org/10.3923/ajbs.2011.90.95.

Mitchell, M.A., Kettlewell, P.J., 2004. Transport of chicks, pullets and spent hens. Welfare of the Laying Hen. CABI, p. 361.

Mitchell, M.A., Kettlewell, P.J., 2009a. Transport of chicks, pullets and spent hens. Welfare of the Laying Hen. In: Proceedings of the Papers from the 27th Poultry Science Symposium of the World's Poultry Science Association (UK Branch), Bristol, UK, July 2003, pp. 361–374. ⟨https://doi.org/10.1079/9780851998138.0361⟩.

Mitchell, M.A., Kettlewell, P.J., 2009b. Welfare of poultry during transport–a review. In: Proceedings of the Poultry Welfare Symposium, pp. 90–100.

National Research Council, 2006. Guidelines for the Humane Transportation of Research Animals. National Academies Press.

Nicol, C.J., Pope, S.J., 1996. The maternal feeding display of domestic hens is sensitive to perceived chick error. Anim. Behav. 52 (4), 767–774.

Nordquist, R.E., Heerkens, J.L.T., Rodenburg, T.B., Boks, S., Ellen, E.D., van der Staay, F. J., 2011. Laying hens selected for low mortality: behaviour in tests of fearfulness, anxiety and cognition. Appl. Anim. Behav. Sci. 131 (3–4), 110–122.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2018. R Core Team. nlme: Linear and Nonlinear Mixed Effects Models. ⟨https://cran.rproject.org/package=nlme⟩.

R Core Team, 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria (3.5.0). ⟨http://www.r-project.org/⟩.

R Studio Team, 2020. RStudio: Integrated Development Environment for R. RStudio (version 1.3.1056). ⟨http://www.rstudio.com/⟩.

Rodenburg, T.B., Van Hierden, Y.M., Buitenhuis, A.J., Riedstra, B., Koene, P., Korte, S.M., Van Der Poel, J.J., Groothuis, T.G.G., Blokhuis, H.J., 2004. Feather pecking in laying hens: new insights and directions for research? Appl. Anim. Behav. Sci. 86 (3–4), 291–298.

Shaffer, J.P., 1995. Multiple hypothesis testing. Annu. Rev. Psychol. 46 (561–584) https://doi.org/10.1146/annurev.ps.46.020195.003021.

Shimmura, T., Hirahara, S., Azuma, T., Suzuki, T., Eguchi, Y., Uetake, K., Tanaka, T., 2010. Multi-factorial investigation of various housing systems for laying hens. Br. Poult. Sci. 51 (1), 31–42. https://doi.org/10.1080/00071660903421167. ⟨http://www.informaworld.com/⟩.

Valros, A., Vuorenmaa, R., Janczak, A.M., 2008. Effect of simulated long transport on behavioural characteristics in two strains of laying hen chicks. Appl. Anim. Behav. Sci. 109 (1), 58–67. https://doi.org/10.1016/J.APPLANIM.2007.02.007.

Venables, W.N., Ripley, B.D., 2002. Random and mixed effects. Modern Applied Statistics With S-Plus. Springer, pp. 271–300.

Versace, E., Fracasso, I., Baldan, G., Dalle Zotte, A., Vallortigara, G., 2017. Newborn chicks show inherited variability in early social predispositions for hen-like stimuli. Sci. Rep. 7 (1), 1–7.

Wang, P., Sun, Y., Fan, J., Zong, Y., Li, Y., Shi, L., Isa, A.M., Wang, Y., Ni, A., Ge, P., Jiang, L., Bian, S., Ma, H., Jiang, R., Liu, X., Chen, J., 2020. Effects of monochromatic green light stimulation during embryogenesis on hatching and posthatch performance of four strains of layer breeder. Poult. Sci. https://doi.org/10.1016/j.psj.2020.06.074.

Weeks, C.A., 2014. Poultry handling and transport. In: Grandin, T. (Ed.), Livestock Handling and Transport, fourth ed. CABI Publishing, Oxon, UK, pp. 378–398. https://doi.org/10.1079/9781845932190.0295. Chapter 20.

Winter, J., Toscano, M.J., Stratmann, A., 2021. Piling behaviour in Swiss layer flocks: description and related factors. Appl. Anim. Behav. Sci. 236, 105272 https://doi.org/10.1016/j.applanim.2021.105272.

Xin, H., Lee, K., 1997. Physiological evaluation of chick morbidity during extended posthatch holding. J. Appl. Poult. Res. 6 (4), 417–421. https://doi.org/10.1093/japr/6.4.417.

Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1 (1), 3–14. https://doi.org/10.1111/j.2041-210x.2009.00001.x.