



# Effects of hatching on-farm on behaviour, first week performance, fear level and range use of organic broilers

Camilla Toldevar Jessen<sup>a</sup>, Leslie Foldager<sup>a,b</sup>, Anja B. Riber<sup>a,\*</sup>

<sup>a</sup> Department of Animal Science, Faculty of Technical Sciences, Aarhus University, Blichers Allé 20, P.O. Box 50, DK-8830, Tjele, Denmark

<sup>b</sup> Bioinformatics Research Centre, Faculty of Natural Sciences, Aarhus University, C.F. Møllers Allé 8, DK-8000, Aarhus C, Denmark

## ARTICLE INFO

### Keywords:

Behaviour  
Broiler  
Fear  
On-farm hatching  
Performance  
Welfare

## ABSTRACT

Hatching on-farm is an alternative to traditional hatching in the hatchery where incubated eggs are placed on the farm on embryonic day 18 for hatching to take place. Thus, several hatchery procedures and transport of newly hatched chicks are avoided, and chicks have access to feed and water immediately after hatching. In the present study, the aim was to examine the behaviour, first week performance, fear level and range use of hatching slower-growing organic broilers on-farm (OF). Chicks hatched in the hatchery (HC) and transported to the farm were used for comparison. The study included six flocks of both treatments, each consisting of approximately 3600 mixed-sex Hubbard JA57 ColorYield broilers, housed with veranda and range access. Compared to HC, the body weight was consistently higher for OF chicks at 0 h, 24 h, 48 h and D7 relative to arrival of HC chicks ( $P < 0.001$ ). Feeding was more frequently observed in OF than HC chicks at 11 h and 35 h ( $P < 0.024$ ). Generally, more HC than OF chicks were drinking ( $P < 0.001$ ), and more OF than HC chicks were resting during the first 23 h ( $P < 0.016$ ). The crop content differed between treatments at 6 h, 12 h, 24 h and 36 h, but not at 48 h: At 6 h, OF chicks had higher odds of having water (odds ratio (OR) = 2.45;  $P < 0.001$ ) and lower odds of having feed in the crop (OR = 0.16;  $P < 0.001$ ). In addition, they had higher odds of having an empty crop at 6 h (OR = 3.01;  $P < 0.001$ ), 12 h (OR = 1.56;  $P = 0.018$ ) and 36 h (OR = 2.56;  $P < 0.001$ ). Reduced fear of humans was found during the first week for OF chickens when assessed in a stationary person test ( $P < 0.030$ ). OF chickens also tended to express less general fear than HC chickens in a novel object test (D7, D28, D53 and D60;  $P = 0.052$ ). However, contrary to expected, the reduced general fearfulness expressed by OF chicks in the novel object test did not result in increased veranda and range use ( $P = 0.92$  and  $P = 0.45$ , respectively). To conclude, on-farm hatching of slower-growing broilers appears to benefit animal welfare as, during early age, it reduces fearfulness and allows for more resting and feeding, likely being the cause of increased body weight.

## 1. Introduction

As part of the standard production scheme, newly hatched broiler chicks are transported from the hatchery to the farm within one to three days post-hatch. Handling, sexing, vaccination and crating at the hatchery, followed by transportation and unloading are stressors, which are prevalent experiences in the chick's early life (Mitchell, 2009; EFSA, 2011; Hedlund et al., 2019). In addition, several other stressors are associated with hatching in a hatchery, including hatching in continuously dark and noisy incubators and being deprived of feed and water. Continuous darkness increases fear responses (Archer and Mench, 2014), while delayed feed intake increases the risk of hypothermia

(Willemsen et al., 2010) and negatively affects a number of parameters, including fear response, body weight, mortality, immune system as well as development of intestines and muscles (Shira et al., 2005; Simon et al., 2015; Hollemans et al., 2018; Uni and Ferket, 2019).

Several studies, examining the effects of transport on newly hatched chicks, find adverse effects on performance (e.g. loss of body weight (Bergoug et al., 2013; Jacobs et al., 2016)) and stress physiology (e.g. increased plasma corticosterone (Jacobs et al., 2017)). In addition, transport has been linked with greater latency to righting in a tonic immobility test, which points to increased fearfulness in transported chicks (Mancinelli et al., 2018). Some studies have shown that the negative impact of transportation depends on the duration of transport

Abbreviations: OF, on-farm hatched; HC, hatchery hatched.

\* Corresponding author.

E-mail address: [anja.riber@anis.au.dk](mailto:anja.riber@anis.au.dk) (A.B. Riber).

<https://doi.org/10.1016/j.applanim.2021.105319>

Received 1 February 2021; Received in revised form 30 March 2021; Accepted 31 March 2021

Available online 5 April 2021

0168-1591/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

(Bergoug et al., 2013; Jacobs et al., 2017), whereas others find no or only minor effects of duration (Valros et al., 2008; Jacobs et al., 2016).

In the organic farming principles, animal welfare is given priority. Specifically, the EU regulation on organic farming states that ‘organic production shall establish a sustainable management system for agriculture that respects high animal welfare standards and in particular meets animals’ species-specific behavioural needs’ (Art. 3 in EC (2007)). One way to pursue this could be to change the hatching location from the hatcheries to the farms by placing the incubated eggs on the farm on embryonic day 18 (E18). This way, several hatchery procedures and transport of newly hatched chicks can be avoided. Additionally, by hatching on-farm, the chicks have access to feed and water immediately after hatching. Indeed, higher first-week body weight has been found for fast-growing broilers hatched on-farm as compared to broilers hatched in hatcheries (de Jong et al., 2019, 2020; Souza da Silva et al., 2021). Furthermore, Giersberg et al. (2020) showed that on-farm hatched broiler chicks displayed signs of being less stressed, as they vocalised less in a novel environment test on day 1 (D1) compared to hatchery-hatched chicks.

Whether new practices, such as hatching on-farm, benefit animal welfare, can be evaluated in comprehensive welfare assessments. One approach, recommended by Fraser (2008), is to include aspects of three welfare concerns for animal welfare: a) the affective states of animals; b) the ability of animals to lead reasonable natural lives and c) the basic animal health and functioning. Based on the research reviewed above, fearfulness appears to be a relevant affective state to assess in relation to effects of hatching location. Fearfulness is defined as the propensity to experience fear or anxiety, with fear being a reaction to the perception of actual danger (Boissy, 1995). It can be either general or specific, where the general fearfulness is a personality or temperament trait defining the general susceptibility of an individual to react to a variety of potentially threatening situations, whereas the latter is based on fear of specific stimuli, e.g. humans (Boissy, 1995; Waiblinger et al., 2006). For poultry, general fearfulness has been suggested to be negatively correlated with one of the most fundamental elements in the organic mindset, i.e. the use of the range (Stadig et al., 2017), which is generally low in broilers (e.g. Dawkins et al., 2003).

The aim of the present study was to do a comprehensive welfare assessment of slower-growing organic broilers hatched on-farm by using the approach suggested by Fraser (2008). Chicks hatched at the hatchery and transported to the farm were used as a control group. The impact of hatching location on behaviour, first week performance, fear level and range use are reported here, whereas hatchability, chick quality, litter quality and a range of clinical welfare indicators and slaughter parameters will be reported in Jessen et al. (*unpublished*). As previous studies have focussed on fast-growing broilers without veranda or range access, we were particularly interested in examining whether hatching on-farm would increase use of the veranda and range, expectedly through a decrease in general fearfulness arisen from a potentially less stressful early postnatal life.

## 2. Materials and methods

The data were collected from August 2019 to January 2020 on a private organic broiler farm in Northern Jutland, Denmark. The study was conducted in accordance with Danish legislation and guidelines regarding organic farming (Landbrugsstyrelsen, 2020).

### 2.1. Animals and housing

The study consisted of six consecutive replicates of two treatment groups of organic broiler chickens housed according to the guidelines for organic farming. The two treatment groups were placed in different sections of a barn, and each treatment group consisted of approximately 3600 mixed-sex Hubbard JA57 ColorYield broiler chickens, which is a slower-growing hybrid with a growth rate of 35–42 g/day (Hubbard, 2021). Males were slaughtered on D52–56, leaving the females for

approximately another week until they were slaughtered on D60–63. The stocking density at D0 was 9.8 birds/m<sup>2</sup> with approximately 19.0 kg/m<sup>2</sup> on the day where the males were slaughtered.

The farm, hosting the study, consisted of two buildings, each housing two barns (Fig. 1). The buildings were identical in construction and placed parallel to each other, approximately 290 m apart with a range for the chickens in between. Likewise, the four barns were identical in design. Within each building, a common entrance room was placed in the middle, providing access to the two barns. Each barn was divided in two sections (Fig. 1): one section near the common entrance room (Section 1) and one section in the back of the barn (Section 2) closest to the barn gate. Artificial light was provided and windows allowed entry of natural light. During E18½–D2 light was on 24 h/day, whereas from D3 the light was switched off at 22:00 h and on at 06:00 h, i.e., 16 h light/8 h dark, with 15 min dusk and dawn periods contained in the light hours.

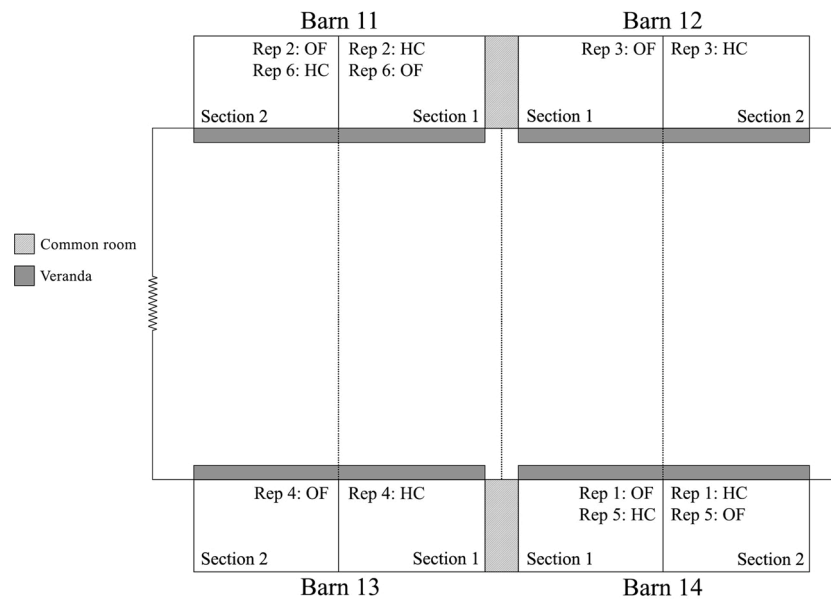
There were three water lines and two lines of feed troughs running along the two sections of the barn, through the section divider, outlining six distinct corridors (Fig. 2). In each section, there were 249 water nipples and 50 feed troughs. Twelve straw bales were allocated per section of which ten were placed in areas where behavioural observations of resting took place (Fig. 2). Pinewood shavings were used as bedding material in both sections during replications 1, 2, 3 and 6, whereas straw pellets were used in both sections in replications 4 and 5. Feed was spread on paper under each line of feed troughs to encourage feeding at placement/hatch.

On the long side of each section, there were three pop-holes (L × H: 3.5 m × 0.5 m, each) to a covered veranda (L × W: 20.4 m × 4 m). First day of access to the veranda and range was on D35 of age. The range (W × L: approximately 85 m × 290 m, Fig. 1), placed between the two buildings, was split into one elongated outdoor area for each of the sections in the barns, i.e., eight in total. The outdoor areas were separated with a wire-mesh fence (H: 1 m) to keep chickens from different sections from mixing, and the outer perimeter consisted of an approximately 2 m high electrical fence (PIT Hegn, Østjysk Hegn ApS®, Ringe, Denmark) to keep predators out. The eight sections of the outdoor area were of approximately similar overall composition and dimensions (L × W: 145 m × 20–22 m). The first part of the outdoor areas was sand (3 m), which was followed by gravel for the next 2–3 m. The next part consisted of combinations of sparse, tall and dense vegetation and persisted for 5–6 m. The remaining part was dominated by grass and 1–3-year-old poplar trees.

### 2.2. Treatments

The study included two treatments where one treatment consisted of chicks hatched at a hatchery and transported as day-old to the farm (HC), and the other consisted of chicks hatched on-farm (OF) using the One2Born system (One2Born, 2021). For each replicate, the eggs of both OF and HC chicks were from the same parent flock and were placed simultaneously in the same storage room and later in the same incubator at the hatchery (DanHatch A/S, Vrå, Denmark). Hence, the parental origin and conditions during storage and incubation conditions were identical for both treatments until D18 of incubation. Eggs from both treatments were candled on E18, just before transportation of the OF eggs to the barn and the movement of the HC eggs to the hatcher at the hatchery. The OF eggs were placed in one section of one of the barns on E18½, and the day-old HC chicks, arriving on D0 (corresponding to E21), were placed in the other section. During the hatching on-farm, the eggs were placed in approximately 77 egg trays (‘hatchholders’) in a restricted area (approximately 10 m × 3.8 m; Suppl. Fig. S1). The hatchholders resemble traditional egg trays, but they hold 50 eggs each and are designed with multiple venturi and convection channels, so a natural airflow is facilitated. When the hatchholders containing the eggs were placed, feed was provided in between.

After hatching at the hatchery, the HC chicks were sorted, i.e.,



**Fig. 1.** A schematic illustration of the farm (not to scale) hosting the study, showing the two buildings, each consisting of two barns divided into two sections. The ranges, the verandas and the placement of the two treatments in the different replicates are indicated.

second-grade chicks were discarded, and first-grade chicks were vaccinated and then transported for 1 h to the farm. From hatch to placement at the farm, 5–25 h had passed for the HC chicks, depending on whether they hatched early or late (personal communication, production manager Kim Risgaard Larsen, DanHatch, Vrå, Denmark). Just before placement of the HC chicks on D0, the successfully hatched OF chicks were sorted by the farmer to ensure that second-grade chicks were removed like the standard procedure at the hatchery. Chicks with beak and/or head abnormalities (e.g. cross beak, eyes missing, exposed brains), navel infections, lameness, ectopic viscera, etc. were culled, and unhatched eggs were counted, removed and placed in a gas chamber to ensure proper killing of chicks potentially still alive in the eggs. To ensure similar group sizes of the two treatment groups, any surplus OF chicks were killed. The remaining OF chicks were placed in boxes of approximately 50 chicks per box and moved to the part of the barn where vaccinations took place immediately thereafter. While the chicks were vaccinated, the hatchholders and material used to restrict the area during hatching were removed, allowing OF chicks access to the entire section upon completion of vaccination.

The chicks were vaccinated according to the common vaccination program for slower-growing organic broilers by a veterinarian and two assistants. Vaccines for Marek's disease and Gumboro were injected in the necks of the chicks in a fast and semi-automatic way, using a machine identical to the one used in the hatchery. Vaccines against coccidiosis and infectious bronchitis were applied using a spray cabinet. After drying for 15–20 min, the chicks were released. The HC chicks went through similar vaccination procedures at the hatchery.

Two of the barns were used for two replicates each, and the other two for one replicate each. The replicates were placed consecutively with 14 days in between each replicate, with the exception that replicates 3 and 4 were separated by 28 days. To minimise location bias between the two sections, the two treatment groups were placed in alternating sections in the barns for each replication such that each treatment was placed three times in each of the two sections (Fig. 1).

### 2.3. Data collection

Throughout the rearing period, data on several parameters were collected as described in the following paragraphs (Table 1). The hours indicated for different types of data collection are in relation to

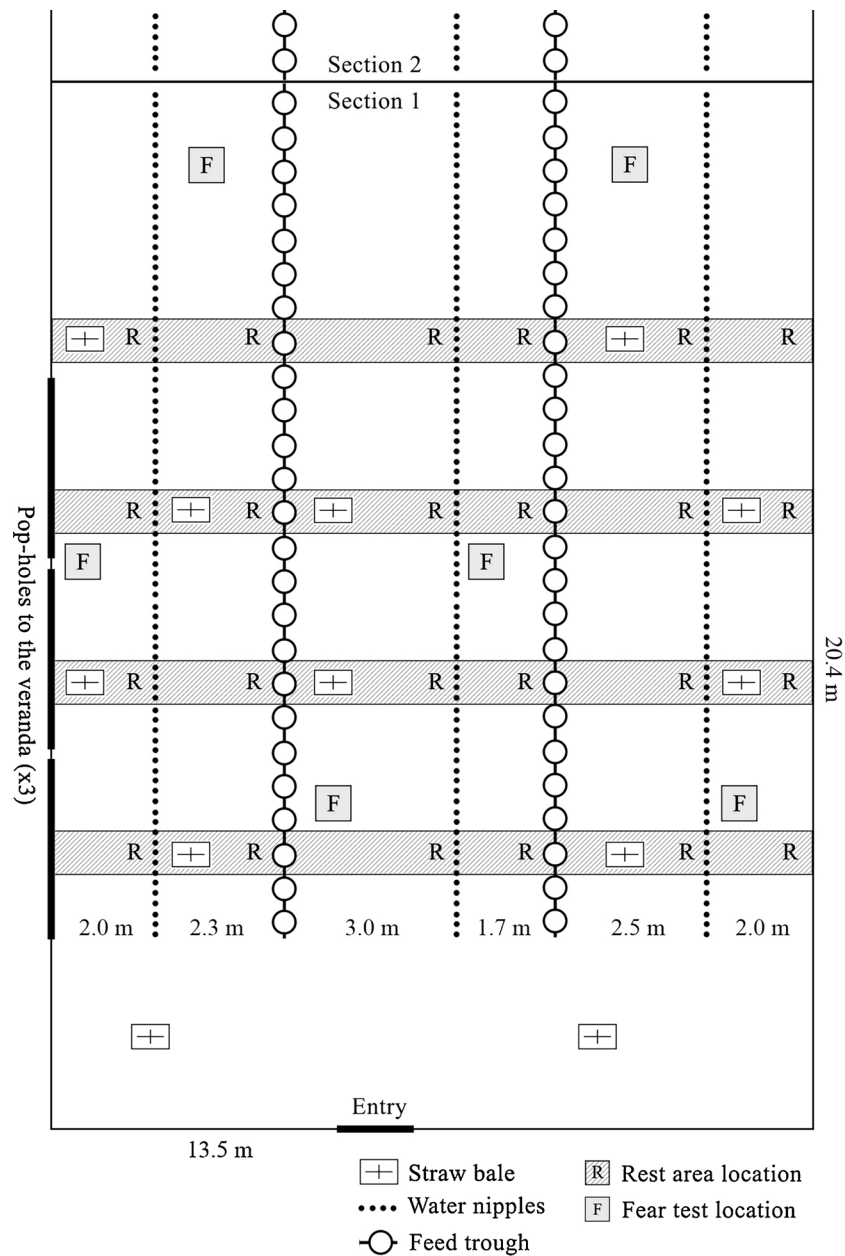
placement of HC chicks. Up until slaughter of males on D52–56, data collection was done on mixed-sex groups, whereas data collected after D56 were from female chickens. Only one observer was involved in the assessment of chick quality, fearfulness and use of veranda and outdoor area. For the data collection on resting, drinking and feeding behaviour, five, six and six observers were involved, respectively, whereas five observers were involved in data collection on body weight and crop content, but only one observer did the data collection for both treatment groups within a given age and replication. All observers had been trained together prior to the commencement of the study.

#### 2.3.1. Behavioural observations

The number of chicks performing feeding, drinking and resting behaviour within specified observation areas (see below) was determined using instantaneous scan sampling (once for each observation area, done consecutively) at –3 h, 5 h, 11 h, 23 h, 35 h, 47 h and on D7. The observer walked slowly down the middle of the barn for optimal view of all corridors of the barn, stopping when counting and counting as far ahead as possible to minimise disturbance. The observer always started in section 1 with the resting behaviour. When the resting observations were done in section 1, the observer would move to the other section, while two observers walking together, one doing the drinking behaviour, the other doing the feeding behaviour, would start the observations in section 1 in the same manner as described for the resting behaviour. The total time observers were present in the barn for the behavioural observations was approximately 40–45 min. The observation done at –3 h only concerned the OF chicks and was done merely for descriptive purposes.

For every fourth feed trough, the number of chicks feeding on the paper or in the trough within a predefined area was counted. The observational area ranged between the middle of the gaps to the neighbouring troughs on each side of the observational trough. The number of chicks manipulating every tenth water nipple or cup underneath was counted. For a chick to be resting, it had to sit motionless with its head dropped. All chicks resting within a predefined area were counted. Four areas were chosen from each of the six corridors, resulting in 24 predefined areas in total for each section (Fig. 2).

When the area of the OF chicks was restricted (–3 h), the number of chicks feeding around the hatchholders (within a chick length) was counted. Thirty hatchholders were chosen in a zigzag pattern to cover



**Fig. 2.** A schematic illustration of a section in the barn (not to scale), showing the distribution of straw bales and feed and water lines. The approximate locations for the data collection on resting (described in section 2.3.1) and performance of fear tests (described in section 2.3.4).

the whole area as evenly as possible. Furthermore, the number of OF chicks manipulating any of the nipples in the restricted area was counted. Resting chicks were counted on and around (within a chick length) 12 hatchholders. The hatchholders were chosen in an alternating manner to cover the area evenly, i.e., every second hatchholder was chosen from lines three and five (six lines in total), starting with the first and second hatchholder, respectively.

### 2.3.2. Crop content

The crop content was registered by external palpation of live chicks from both treatments at 6 h, 12 h, 24 h, 36 h and 48 h when the behavioural observations were completed, i.e. together with body weight, crop content would be the last data collected on days where it coincided with other activities (Table 1). In addition, it was done for the OF chicks during the chick quality assessment at 0 h. In each treatment, 10 chicks from 10 different areas were picked up and placed in a bucket. An observer would gently palpate the crop to determine if the chick had

accessed both feed and water (enlarged and soft crop containing detectable crumbles; score 1), only water (enlarged and soft crop, no detectable crumbles; score 2), only feed (enlarged, hard and edged crop, the texture of the crumbles was apparent; score 3) or the crop was empty (score 4). The lower the score, the better the chick had performed, and thus water intake was considered more important than only feed intake (Henriksen et al., 2016).

### 2.3.3. Body weight

Manual weighing of 100 chicks (scale: CJ-6200CE, VIBRA SHINKO DENSHI CO., LTD©, Tokyo, Japan; precision  $\pm 0.1$  g) was done twice for HC chicks (prior to and after transport) and once for the OF chicks (upon placement of HC chicks). Registration of body weight was subsequently repeated at 24 h and 48 h and on D7. To minimise the risk of panic and injuries during catching on D7, the artificial light was switched off immediately beforehand, leaving only the natural light. For each time point, 10 chicks from 10 different areas ( $n = 100$ ) in each treatment



**Table 1**

Overview of the data collection arranged in order according to age of the chicken (relative to placement of HC chicks) when the data collection was performed.

Age	Parameter					
	Behaviour	Chick quality	Body weight	Crop content	Fear tests	Veranda and outdoor use
-3 h	x* (only OF)	x (only HC)	x (only HC)			
0 h		x	x	x* (only OF)		
5 h	x					
6 h				x		
11 h	x					
12 h				x		
23 h	x				x (only SPT)	
24 h			x	x		
35 h	x					
36 h				x		
47 h	x					
48 h			x	x		
D7	x		x		x	
D28					x	x
D35						x
D42						x
D53					x	x
D60					x	

\* Data collection only concerned the OF chicks and was done merely for descriptive purposes.

were weighed.

#### 2.3.4. Fear

Fearfulness was assessed on D1, D7, D28 and the day before slaughter of males (D51–55, termed D53 hereafter) and females (D59–62, termed D60 hereafter), respectively. Two tests were applied on each test day, a stationary person test (SPT) and a novel object test (NOT), except for D1 where only SPT was performed. Both fear tests were based on the procedures described in Brantsæter et al. (2017). Furthermore, all observations were done in real time in the barn, and therefore the perimeters used (see below) were estimated by the observer performing the test.

On those days when the fear tests coincided with the behavioural observations, the observer would always perform tests of fearfulness before engaging in observations of resting, and the two types of data collection were separated by approximately 1 h. Both tests were performed in six locations in each section of the barn: one in each of the six corridors at different distances to the divider of the two sections (Fig. 2). The tests were performed in approximately the same locations on all test days for all treatment groups. However, if a location was completely devoid of any chickens, the test was performed at a nearby location with chickens present. The observer always started in section 1.

In SPT, the observer walked slowly through the section, stopped at each of the six predefined locations. At each location, the number of chickens within a distance of approximately 2 m and 25 cm, respectively, from the observer (360°) was determined every 10 s for 2 min. This was done without moving the feet, but by quietly twisting the upper body. On D28, D53 and D60, due to the chicks' larger size, chickens were counted as being within the distance when the chicken's clavicle had crossed the perimeter. A chicken with its back turned to the observer was still counted as within distance if the tail feathers crossed the perimeter. The observer also noted the latencies for three chickens to cross within the distance of approximately 2 m and 25 cm, respectively.

Immediately after performing SPT at a given location, the observer performed NOT at the same location. Following placement of the novel object on the floor, the observer stepped back approximately 3 m. Every 10 s for a total of 2 min, the observer counted the number of chickens within a distance of approximately 25 cm from the edge of the object.

Additionally, the latencies for three chickens to cross within a distance of 2 m and 25 cm, respectively, from the object were noted. Different objects were used on the different days of testing, each with a size suitable to the size of the chickens at the age of performing the test. The objects used were a yellow highlighter pen (D7), a classic Coca Cola can (33 cl; D28), a basketball (D53) and a metal wire mesh paper bin (D60; Suppl. Fig. S2).

#### 2.3.5. Veranda and range use

The numbers of chickens present in the veranda and in the range were registered on the first day of access (D35), one week later (D42) and on the day before slaughter of males (D53). Observations were done in the morning (09:00–10:30 h), at noon (12:15 h) and in the afternoon (14:00–15:00 h). The exact time of observation in the morning and afternoon sessions varied due to the decreasing day length in the study period.

After entering the veranda (only accessible from the range), the observer would go to the corner of the veranda of the target section and wait 5 min for the chickens to get accustomed to the presence of a human. While waiting, the temperature, wind speed and humidity in the veranda were measured with a pocket weather meter at approximately 1 m height (Kestrel 3000, Kestrel Meters, Boothwyn, Pennsylvania, USA). Chickens standing in the pop-hole opening were not included in the counting. After counting the chickens in one section, the process was repeated in the other section.

After finishing the observations for the verandas in both sections, the observer went into the range of the first section. Standing approximately 10 m from the veranda by the fence, the observer waited 5 min for the chickens to get accustomed to the presence of a human. While waiting, the temperature, wind speed and humidity were once again measured. The range had beforehand been marked with labels, indicating the distance to the veranda for every 10 m. Slowly the observer walked in the middle of the range, counting the number of chickens within the first 10 m, 10–20 m, 20–30 m and > 30 m from the veranda. After trailing back to the veranda, the observer repeated the process for the range of the other section.

#### 2.4. Statistical methods

Analyses were done using R 4.0.0 software (R Core Team, 2020) and a statistical significance level of 0.05. P-values between 0.05 and 0.10 are reported as tendencies. *Post hoc* pairwise comparisons were performed for significant factors with p-values adjusted to control the false discovery rate (FDR). Estimated marginal means, standard errors and pairwise comparisons were back-transformed to the original scale, when relevant. Supplementary Statistical Methods gives further details on the statistical analyses presented more briefly here.

Feeding behaviour was analysed with a zero-inflated negative binomial generalised linear mixed effects model (GLMM). The model included the factors treatment and age (5 h, 11 h, 23 h, 35 h, 47 h and 168 h (D7)) and their interaction as fixed effects for the conditional mean component, and age for the zero-inflation part. Dispersion was allowed to depend on treatment, age and their interaction. Drinking behaviour was analysed using a zero-inflated Poisson GLMM. The same model was used for fixed effects and zero-inflation components; including treatment and age additively. Test of these factors was carried out jointly and standard error of the combined mean was obtained by ordinary nonparametric bootstrapping. Resting behaviour was analysed using a negative binomial GLMM with treatment, age and their interaction as fixed effects and for the dispersion model. Feeding, drinking and resting models included replication and observer as random effects, results are presented as rates, i.e., mean number of chicks doing the behaviour per observation area or water nipple and comparisons are shown as rate ratios (RR). Behaviours by OF chicks -3 h relative to arrival of HC chicks were estimated with replication as random effect.

Crop content scores were analysed by multinomial logistic regression

with replication and observer added as nuisance factors using neural networks. Crop score 1 was used as reference category, and the model included treatment, age and their interaction as explanatory factors. Comparisons are shown as odds ratios (OR), with odds being probabilities for each of crop scores 2, 3 and 4 versus the probability of crop score 1. Crop content scores at 0 h for the OF chicks were quantified as the mean and standard error of score proportions observed in each replication.

Body weight was analysed with a normal linear mixed effects model (LMM). The model was additive in the fixed effect factors of treatment and age, included replication as random effects, and had age-dependent variances.

The following was used for the SPT. For each replication, treatment and age combination, the average of the 12 counts was determined at each of the six locations. Ages considered for SPT were D1, D7, D28, D53 and D60. For the 25 cm SPT, only two locations on D7 were different from zero for HC and none for OF and, consequently, D7 had to be omitted. The aggregation of the results obtained in the NOT was done equivalently but only for a distance of 25 cm. Ages considered for NOT were D7, D28, D53 and D60. For the 25 cm SPT and NOT, these average counts were analysed by a Delta-lognormal approach (Aitchison, 1955; Fletcher, 2008). Replication was included as random effect. For the 2 m SPT, the average counts (Y) was modelled with a LMM on  $\log(Y + 1)$  to avoid undefined transformation of zeroes. This model included the same fixed and random effects as the 25 cm SPT.

Latency to approach for SPT and NOT was modelled by a mixed effects Cox proportional hazards regression with right censoring after 120 s and replication as random effect. SPT 25 cm had a very high percentage of right censored observations (72–100 %) and was not considered further. The percentage of right censoring in NOT 25 cm was 61–94 %. Since age tended to violate the proportionality assumption, age stratified mixed effects Cox regressions were applied to complement assessments of treatment difference for 2 m SPT and NOT. Treatment and age (only treatment when stratifying on age) were fixed effects, and significance of their interaction was furthermore tested. Comparisons are presented as hazard ratios (HR). To quantify average latency time while taking the right censoring into account, a Gaussian parametric survival regression with clustering on replication was applied.

The use of veranda and range, respectively, was analysed with a negative binomial GLMM that included replication as random effect and  $\log(\text{number of chickens})$  as offset, and had dispersion modelled by age (veranda) and treatment and age (range). The following fixed effects were investigated: treatment, age, humidity, temperature and wind speed. None of the two-way interactions among these were found to be statistically significant. Wind speed at the veranda was zero for 78 % of the samples and not considered further. On each of the sampling days, measurements were done three times; morning, noon and afternoon. The effect of time of day was examined, but it had no significant influence. The aggregated total from the four counting zones was used for the analyses. Comparisons are shown as rate ratios (RR) and means are given as rates per 1000 chickens.

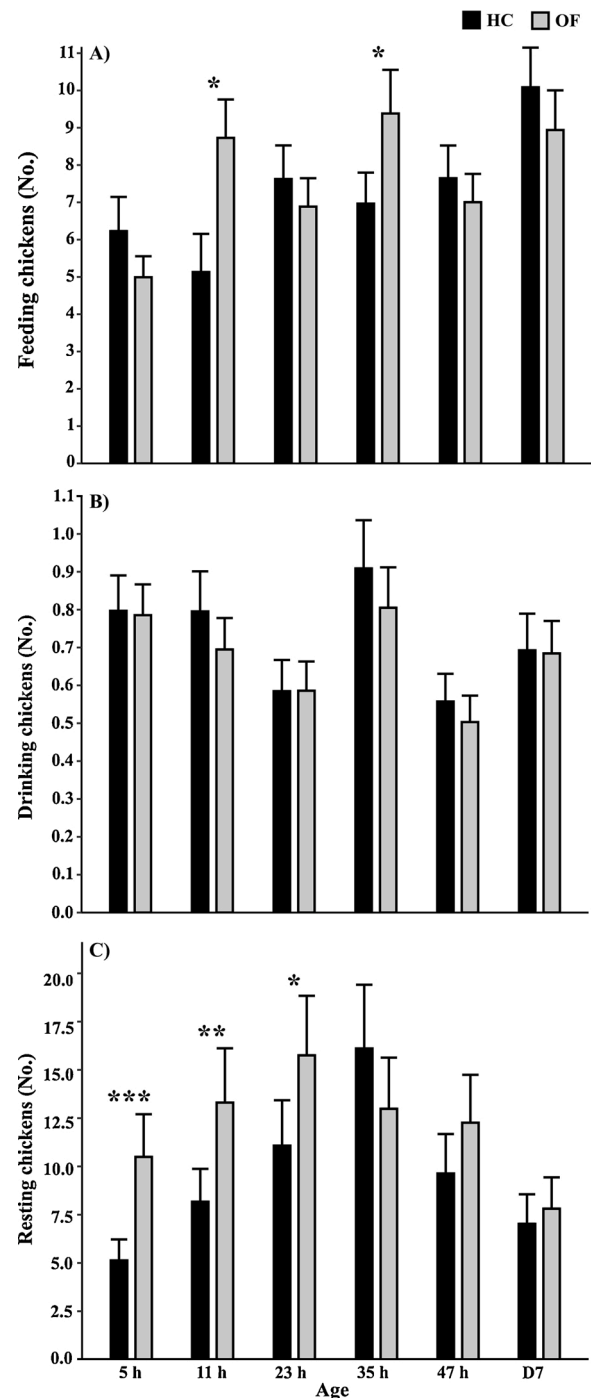
## 2.5. Ethical note

The experiment was carried out according to the guidelines of the Danish Animal Experiments Inspectorate with respect to animal experimentation and care of animals under study.

## 3. Results

### 3.1. Behavioural observations

There was a significant interaction between treatment and age for the mean number of chicks feeding per observation area ( $\chi^2_5 = 17.4$ ,  $P = 0.004$ ; Fig. 3A). There were more feeding OF chicks at 11 h and 35 h



**Fig. 3.** The number of feeding (A), drinking (B) and resting (C) chicks per observation area/nipple at the ages 5 h, 11 h, 23 h, 35 h, 47 h and D7. OF = on-farm hatched chicks, HC = hatchery chicks. The estimates presented are back transformed means  $\pm$  SE. Where interactions between treatment and age exist, significant differences between treatments are indicated as follows: \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ . NB: For drinking, a significant effect was found for both main factors, treatment and age.

compared to HC chicks ( $\chi^2_1 = 6.90$ ,  $P = 0.023$  and  $\chi^2_1 = 6.57$ ,  $P = 0.026$ ). No differences were found between treatments at any other ages ( $0.47 < \chi^2_1 < 1.46$ ;  $P > 0.40$ ). For the observation made at  $-3$  h, the mean number of feeding OF chicks per observation area was  $2.4 \pm 0.33$ .

The mean number of chicks drinking per observation nipple depended significantly on treatment ( $\chi^2_2 = 19.4$ ,  $P < 0.001$ ) and age ( $\chi^2_{10} = 25.9$ ,  $P = 0.004$ ; Fig. 3B). There were more chicks drinking in the HC

section. The mean number of chicks drinking per nipple was higher at 5 h than at 47 h ( $\chi^2_2 = 10.4$ ,  $P = 0.021$ ), whereas no other ages were significantly different ( $1.01 < \chi^2_2 < 8.00$ ;  $P > 0.11$ ). There were  $0.6 \pm 0.05$  OF chicks drinking per observation nipple at  $-3$  h.

There was a significant interaction between treatment and age on the mean number of chicks resting per observation area ( $\chi^2_5 = 22.2$ ,  $P < 0.001$ ; Fig. 3C). More OF than HC chicks were resting at 5 h (RR = 2.05,  $t_{1694} = 4.76$ ,  $P < 0.001$ ), 11 h (RR = 1.63,  $t_{1694} = 3.14$ ,  $P = 0.006$ ) and 23 h (RR = 1.42,  $t_{1694} = 2.55$ ,  $P = 0.027$ ). No difference between treatments was found for the observations at 35 h, 48 h and D7 ( $t_{1694} \leq 2.55$ ,  $P \geq 0.10$ ). There was a mean of  $22.8 \pm 3.35$  OF chicks resting per observation area at  $-3$  h.

### 3.2. Crop content

There was a significant interaction between treatment and age on crop content ( $\chi^2_{12} = 76.9$ ,  $P < 0.001$ ; Fig. 4). At 6 h, OF compared to HC chicks had greater odds of having only water in the crop (OR = 2.50, 95 %CI: 1.40–4.46;  $\chi^2_1 = 26.7$ ,  $P < 0.001$ ), lower odds of having only feed in the crop (OR = 0.15, 95 %CI: 0.08–0.32;  $\chi^2_1 = 71.0$ ,  $P < 0.001$ ) and greater odds of having an empty crop relative to having both water and feed in the crop (OR = 3.03, 95 %CI: 1.20–7.59;  $\chi^2_1 = 15.4$ ,  $P < 0.001$ ). At 12 h, OF chicks had lower odds than HC chick of having only feed in the crop (OR = 0.61, 95 %CI: 0.29–1.26;  $\chi^2_1 = 4.94$ ,  $P = 0.042$ ) and greater odds of having an empty crop (OR = 1.57, 95 %CI: 0.88–2.80;  $\chi^2_1 = 6.53$ ,  $P = 0.019$ ). No difference between treatments was found for having only water in the crop (OF v. HC OR: 0.99, 95 %CI: 0.58–1.68;  $\chi^2_1 < 0.01$ ,  $P = 0.95$ ). At 24 h, OF chicks had lower odds of having only feed in the crop (OR = 0.18, 95 %CI: 0.06–0.50;  $\chi^2_1 = 29.1$ ,  $P < 0.001$ ). No differences were found between treatments for the odds of having only water in the crop or an empty crop (OF v. HC OR: 1.26 and 1.41; 95 %CI: 0.69–2.27 and 0.33–6.11;  $\chi^2_1 = 1.58$  and  $0.59$ ;  $P = 0.34$  and  $0.51$ , respectively).

At 36 h, OF chicks tended to have higher odds than HC chicks of having only water in the crop (OR = 1.53 (95 %CI: 0.78–3.00;  $\chi^2_1 = 4.30$ ,  $P = 0.076$ ) and an empty crop (OR = 2.55, 95 %CI: 1.06–6.13;  $\chi^2_1 = 12.1$ ,  $P = 0.001$ ). No difference was found between treatments for having only feed in the crop (OF v. HC OR: 0.97 (0.31–3.04);  $\chi^2_1 = 0.01$ ,  $P = 0.93$ ). Lastly, at 48 h, no differences were found between treatments for the odds of having only water, only feed or an empty crop (OF v. HC OR: score 2: 1.10, 95 %CI: 0.61–1.97; score 3: 1.79 (0.38–8.41); score 4: 1.65

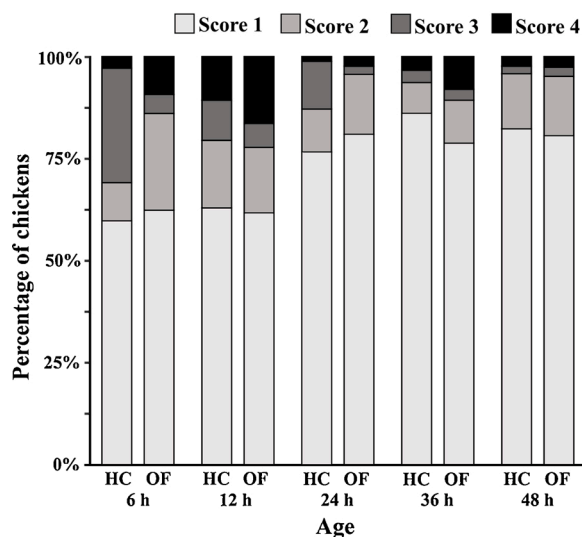


Fig. 4. The frequencies (%) of crop scores (S1-4) of on-farm hatched chicks (OF) and hatchery chicks (HC) at 6 h, 12 h, 24 h, 36 h and 48 h.

(0.43–6.36);  $0.26 < \chi^2_1 < 1.51$ ;  $P > 0.25$ ). At 0 h,  $2.9 \pm 0.9$  % of OF chicks were scored as having both water and feed in the crop,  $6.2 \pm 2.4$  % were scored as having only water in the crop,  $0.8 \pm 0.7$  % were scored as having only feed in the crop and  $90.1 \pm 2.6$  % were scored as having an empty crop.

### 3.3. Body weight

OF chicks had a higher body weight compared to HC chicks ( $F_{1,2793} = 411.5$ ,  $P < 0.001$ ), and as expected, body weight increased with age ( $F_{3,2453} = 8162.5$ ,  $P < 0.001$ ; Table 2). The pre- and post-transport body weight in HC chicks differed ( $F_{1,1186} = 6.53$ ,  $P = 0.011$ ), being  $40.7 \pm 0.60$  g before and  $40.2 \pm 0.60$  g after transport to the rearing farm.

### 3.4. Fear

#### 3.4.1. Stationary person test

The interaction between treatment and age was significant for the number of chickens within 25 cm ( $\chi^2_6 = 21.4$ ,  $P = 0.002$ ; Table 3A) and 2 m ( $F_{4,345} = 2.43$ ,  $P = 0.048$ ; Table 3B) of the stationary person. More OF chicks compared to HC chicks were within 25 cm ( $\chi^2_2 = 17.0$ ;  $P = 0.006$ ) and 2 m ( $t_{345} = 3.14$ ,  $P = 0.004$ ) on D1 and within 2 m on D7 ( $t_{345} = 2.35$ ,  $P = 0.030$ ), but no differences were found at the other ages (25 cm:  $0.07 < \chi^2_2 < 17.0$ ;  $P > 0.18$ ; 2 m:  $-0.38 < t_{345} < 1.58$ ,  $P > 0.15$ ). All treatment groups had chickens, which approached within 2 m in each test on all experimental days.

For latency of three chickens to approach within 2 m of the stationary person, both treatment and age were significant (treatment:  $\chi^2_1 = 7.95$ ,  $P = 0.005$ ; age:  $\chi^2_4 = 39.3$ ,  $P < 0.001$ ; Table 3C). Chickens in OF had a shorter latency to approach (HR = 1.37, 95 %CI: 1.07–1.67). Chicks at D1 approached faster than chickens at all other ages (D7: HR = 2.63 (1.27–4.00),  $\chi^2_1 = 27.5$ ,  $P < 0.001$ ; D28: HR = 2.91 (1.44–4.38),  $\chi^2_1 = 35.2$ ,  $P < 0.001$ ; D53: HR = 1.91 (0.98–2.85),  $\chi^2_1 = 13.8$ ,  $P < 0.001$ ; D60: HR = 2.17 (1.10–3.24),  $\chi^2_1 = 19.2$ ,  $P < 0.001$ ). The only other significant pairwise comparison was longer latency at D28 than D53 (HR = 0.66, 95 %CI: 0.34–0.98,  $\chi^2_1 = 5.73$ ,  $P = 0.033$ ). The age stratified analysis confirmed the shorter latency of OF chickens (HR = 1.37, 95 %CI: 1.10–1.70,  $\chi^2_1 = 7.70$ ,  $P = 0.006$ ).

#### 3.4.2. Novel object test

The number of chickens within 25 cm of the novel object did not differ between OF and HC ( $0.25 \pm 0.03$  v.  $0.26 \pm 0.03$ ;  $\chi^2_2 = 0.12$ ,  $P = 0.94$ ; Table 3D), but an effect of age was found ( $\chi^2_6 = 47.1$ ,  $P < 0.001$ ). Fewer chickens approached within 25 cm on D53 than D60 ( $\chi^2_2 = 8.15$ ,  $P = 0.020$ ), more chickens approached on D7 than D28, D53 and D60 ( $18.2 < \chi^2_2 < 33.7$ ;  $P < 0.001$ ) and more chickens approached on D28 than D53 ( $\chi^2_2 = 10.6$ ,  $P = 0.007$ ). There were no differences between D28 and D60 ( $\chi^2_2 = 1.46$ ,  $P = 0.48$ ).

For the latency of three chickens to approach within 25 cm of the novel object, there was no effect of treatments (OF v. HC: HR = 1.16 (0.61–1.70),  $\chi^2_1 = 0.37$ ,  $P = 0.54$ ; Table 3E). Age showed an overall significant effect ( $\chi^2_3 = 19.2$ ,  $P < 0.001$ ). The latency was lower on D7 than on D53 and D60 ( $\chi^2_1 = 7.55$  and  $11.2$ ,  $P = 0.012$  and  $0.005$ ), and lower on D28 than on D53 and D60 ( $\chi^2_1 = 5.40$  and  $8.89$ ,  $P = 0.030$  and

Table 2

Body weights at the ages of 0 h, 24 h, 48 h and D7 (relative to the arrival of HC chicks) for both treatments. The model estimates are mean  $\pm$  SE.

Age	OF (g)	HC (g)
0h	43.1 $\pm$ 0.55	40.1 $\pm$ 0.55
24h	49.1 $\pm$ 0.56	46.1 $\pm$ 0.56
48h	56.9 $\pm$ 0.58	53.9 $\pm$ 0.58
D7	115.3 $\pm$ 0.72	112.3 $\pm$ 0.72

**Table 3**

Results from the two fear tests, the stationary person test (A-C) and the novel object test (D-F). The model estimates presented are mean  $\pm$  SE, and the Post-hoc pairwise comparisons of OF vs. HC are presented with FDR adjusted p-values.

Stationary person test (SPT)					
	No. chickens, 25 cm	OF	HC	Statistics	
A	D1	0.16 $\pm$ 0.044	0.03 $\pm$ 0.012	$\chi^2_2 = 17.0$ ; $P = 0.0058$	
	D28	0.03 $\pm$ 0.011	0.11 $\pm$ 0.059	$\chi^2_2 = 4.64$ ; $P = 0.18$	
	D53	0.17 $\pm$ 0.062	0.18 $\pm$ 0.064	$\chi^2_2 = 0.07$ ; $P > 0.99$	
	D60	0.08 $\pm$ 0.027	0.11 $\pm$ 0.040	$\chi^2_2 = 2.51$ ; $P = 0.42$	
		<b>No. chickens, 2 m</b>	<b>OF</b>	<b>HC</b>	<b>Statistics</b>
B	D1	22.0 $\pm$ 5.15	12.4 $\pm$ 3.00	$t_{345} = 3.14$ ; $P = 0.0036$	
	D7	3.52 $\pm$ 1.01	2.02 $\pm$ 0.68	$t_{345} = 2.35$ ; $P = 0.0302$	
	D28	6.52 $\pm$ 1.68	7.02 $\pm$ 1.80	$t_{345} = -0.38$ ; $P = 0.78$	
	D53	10.9 $\pm$ 2.67	8.07 $\pm$ 2.03	$t_{345} = 1.58$ ; $P = 0.15$	
	D60	6.97 $\pm$ 1.79	7.29 $\pm$ 1.86	$t_{345} = -0.23$ ; $P = 0.86$	
C		<b>Latency<sup>1</sup>, 2 m (s)</b>	<b>OF</b>	<b>HC</b>	<b>Statistics</b>
	D1	1.6 $\pm$ 1.19	1.5 $\pm$ 0.89	$t_{349} = 0.16$ ; $P = 0.92$	
	D7	15.2 $\pm$ 6.63	37.6 $\pm$ 7.47	$t_{349} = -8.06$ ; $P < 0.0001$	
	D28	20.9 $\pm$ 6.29	28.7 $\pm$ 10.8	$t_{349} = -0.82$ ; $P = 0.62$	
	D53	7.75 $\pm$ 3.89	24.1 $\pm$ 13.4	$t_{349} = -1.29$ ; $P = 0.34$	
D		<b>No. chickens, 25 cm</b>	<b>OF</b>	<b>HC</b>	<b>Statistics</b>
	D7	0.40 $\pm$ 0.138	0.46 $\pm$ 0.146	$\chi^2_2 = 0.97$ ; $P = 0.69$	
	D28	0.23 $\pm$ 0.049	0.31 $\pm$ 0.051	$\chi^2_2 = 2.02$ ; $P = 0.42$	
	D53	0.18 $\pm$ 0.044	0.14 $\pm$ 0.049	$\chi^2_2 = 5.21$ ; $P = 0.12$	
	D60	0.21 $\pm$ 0.056	0.21 $\pm$ 0.035	$\chi^2_2 = 2.51$ ; $P = 0.38$	
E		<b>Latency<sup>1</sup>, 25 cm (s)</b>	<b>OF</b>	<b>HC</b>	<b>Statistics</b>
	D7	137 $\pm$ 19.9	152 $\pm$ 16.6	$t_{279} = -0.70$ ; $P = 0.65$	
	D28	162 $\pm$ 30.2	150 $\pm$ 21.9	$t_{279} = 0.30$ ; $P = 0.91$	
	D53	201 $\pm$ 30.1	200 $\pm$ 32.4	$t_{279} = 0.12$ ; $P = 0.92$	
	D60	193 $\pm$ 19.6	248 $\pm$ 43.3	$t_{279} = -1.64$ ; $P = 0.39$	
F		<b>Latency<sup>1</sup>, 2 m (s)</b>	<b>OF</b>	<b>HC</b>	<b>Statistics</b>
	D7	75.1 $\pm$ 17.5	88.6 $\pm$ 20.7	$t_{279} = -0.86$ ; $P = 0.60$	
	D28	13.3 $\pm$ 2.86	12.9 $\pm$ 3.41	$t_{279} = 0.08$ ; $P = 0.94$	
	D53	17.2 $\pm$ 13.1	30.8 $\pm$ 16.2	$t_{279} = -0.72$ ; $P = 0.60$	
	D60	22.5 $\pm$ 10.7	28.3 $\pm$ 18.8	$t_{279} = -0.65$ ; $P = 0.63$	

<sup>1</sup> The latency for three chickens to approach.

0.009), whereas no differences were found between D7 and D28 or D53 and D60 ( $\chi^2_1 = 0.24$  and  $0.75$ ,  $P = 0.63$  and  $0.46$ ).

For the latency to approach within 2 m of the novel object, a tendency of an effect of treatment was found with a lower latency for OF than HC chickens (HR = 1.29, 95 %CI: 0.96–1.63,  $\chi^2_1 = 3.77$ ,  $P = 0.052$ ; Table 3F). This was not changed by analysis with age stratification ( $\chi^2_1 = 3.56$ ,  $P = 0.059$ ). The ages differed significantly ( $\chi^2_1 = 116.1$ ,  $P < 0.001$ ). The latency for three chickens to approach within 2 m of the novel object was significantly higher on D7 compared with D28, D53 and D60 ( $58.8 < \chi^2_1 < 81.4$ ,  $P < 0.001$ , HR < 0.20) and significantly higher on D28 compared to D53 ( $\chi^2_1 = 5.60$ ,  $P = 0.027$ , HR = 0.64, 95 %CI: 0.33–0.96). The differences in latency were not significant between D28 and D60 or between D53 and D60 ( $1.46 < \chi^2_1 < 1.48$ ,  $P = 0.23$ ).

### 3.5. Veranda and range use

During the observations, the number of chickens being present at the veranda ranged from none to 14.2 % (OF) or 16.4 % (HC) of the treatment group (mean OF vs. HC: 4.5 % vs. 4.3 %). No difference between

treatments was found (RR = 1.01, 95 %CI: 0.86–1.18,  $\chi^2_1 = 0.01$ ,  $P = 0.92$ ), whereas ages differed significantly ( $\chi^2_2 = 63.1$ ,  $P < 0.001$ ) with increasing numbers of chickens in the veranda with age: D42 v. D35 (RR = 2.22 (1.42–3.48),  $t_{98} = 4.32$ ,  $P < 0.001$ ), D53 v. D35 (RR = 4.63 (3.01–7.12),  $t_{98} = 8.69$ ,  $P < 0.001$ ) and D53 v. D42 (RR = 2.08 (1.60–2.71),  $t_{98} = 6.79$ ,  $P < 0.001$ ). The use of the veranda was positively associated with humidity (RR = 1.30 per 10 percentage points, 95 %CI: 1.15–1.48,  $\chi^2_1 = 15.1$ ,  $P < 0.001$ ) and temperature (RR = 1.58 per 5 °C, 95 %CI: 1.23–2.02,  $\chi^2_1 = 12.6$ ,  $P < 0.001$ ). During the observations, the mean humidity was 78.2  $\pm$  2.23 %, the mean temperature 12.4  $\pm$  1.02 °C and the mean wind speed 0.4  $\pm$  0.11 m/s in the veranda.

The same effects as seen for the veranda were seen in the use of the outdoor area, although fewer chickens used the outdoor area than the veranda (OF vs. HC: mean 1.4 % vs. 1.4 %; range 0–13.6 % vs. 0–14.2 %): No difference in use of the outdoor area was found between treatments (RR = 1.17 (0.77–1.80),  $\chi^2_1 = 0.56$ ,  $P = 0.45$ ), whereas an increase in use with age was evident ( $\chi^2_2 = 23.4$ ,  $P < 0.001$ ; D42 v. D35 (RR = 2.79 (0.96–8.11),  $t_{97} = 2.34$ ,  $P = 0.021$ ), D53 v. D35 (RR = 10.4 (3.56–30.2),  $t_{97} = 5.33$ ,  $P < 0.001$ ) and D53 v. D42 (RR = 3.72 (1.72–8.02),  $t_{97} = 4.16$ ,  $P < 0.001$ ). The use of the outdoor area was positively associated with humidity (RR = 1.51 per 10 percentage points, 95 %CI: 1.13–2.02,  $\chi^2_1 = 7.85$ ,  $P = 0.005$ ) and temperature (RR = 1.70 per 5 °C, 95 %CI: 1.03–2.79,  $\chi^2_1 = 4.53$ ,  $P = 0.033$ ). Moreover, there was a negative tendency of wind speed (RR = 0.89 (0.79–1.01) per 1 m/s,  $\chi^2_1 = 3.45$ ,  $P = 0.063$ ) but since eight measurements were missing (7.4 %) we decided to exclude this covariate from the model used for estimation of the other effects. During the observations, the mean humidity was 81.0  $\pm$  2.52 %, the mean temperature 10.6  $\pm$  0.98 °C and the mean wind speed 2.4  $\pm$  0.37 m/s in the outdoor area.

## 4. Discussion

In the present study, clear positive effects of hatching on-farm were found on a range of welfare indicators. These included more resting behaviour, increased body weight, more feeding and reduced fear of humans during early age, whereas a tendency for reduced general fearfulness was found throughout the rearing period. There were indications of HC chicks being exposed to minor dehydration during the hatchery procedures and transport. Unexpectedly, hatching location had no effect on use of veranda and range. To date, this is the first study investigating the effects of on-farm hatching on the welfare of slower-growing broilers with range access. All previous studies performed have examined effects of hatching location in conventional production using fast-growing broiler hybrids. This difference is important to note, as while the concept of hatching on-farm entails omitting hatchery procedures and transport, the day-old slower-growing broilers hatched on-farm in the present study did not completely avoid handling when day-old due to vaccination by injection.

Similar to previous studies (van de Ven et al., 2009; de Jong et al., 2019, 2020; Souza da Silva et al., 2021), we found OF chicks to be heavier than HC chicks at the time of placement of the latter, indicating that hatching on-farm provides a better starting point. It has been proposed that it is likely due to the combination of HC chicks losing body weight during post-hatch holding and transport coupled with the benefits for OF chicks of immediate access to feed and water (Gonzales et al., 2003; Careghi et al., 2005). According to Careghi et al. (2005), a holding time of 25 h before access to feed and water can cause chicks to lose up to 8% of their body weight. In contrast, the late hatchers, experiencing a much shorter holding time, will only lose 1–3 % of their body weight (Careghi et al., 2005). Additionally, OF chicks do not deplete the nutrients of the yolk sac to the same degree as HC chicks (de Jong et al., 2019), suggesting that the hatchery procedures and transport are more energy-demanding than hatching on-farm. One likely explanation to this is energy expenditure on maintaining thermal comfort during transport (Mitchell, 2009). However, the HC chicks in the present study only lost



1.23 % of their body weight during post-hatch holding and transport, probably due to the relatively short duration (5–25 h). Part of the weight loss causing the difference between HC and OF chicks was likely due to dehydration during the deprivation period of the HC chicks, which may explain why more HC chicks were observed drinking during the behavioural observations.

The present study distinguishes itself from previous studies of on-farm hatching by investigating the development in body weight, crop content and behaviour intensely over time during the first 48 h, revealing some interesting time-dependent differences between OF and HC chicks. For instance, more resting was found in OF than HC chicks during the first 23 h. At –3 h before arrival of HC chicks, resting was the main activity performed by the OF chicks, whereas only few were engaged in feeding and drinking, which was confirmed by the finding that 90.1 % had an empty crop at 0 h. These results may have two explanations (see the following paragraphs), working either separately or, more likely, in combination.

Firstly, [de Jong et al. \(2016\)](#) found fast-growing chicks hatching on-farm to start feeding 2.5 h (range 0.5–9 h) and drinking 5 h after hatching, and it has been shown that in general the amount of feed ingested by broilers during the first 24 h is small (1.5 g; [Pinchasov and Noy, 1993](#)). Thus, OF chicks may have ingested a small amount of feed shortly after hatching, that is, for the most part, prior to our observations at –3 h. Then, the disturbances of OF chicks due to the sorting and vaccination procedures performed in the hours immediately before arrival of HC chicks negatively affected the possibilities of feeding, drinking and resting behaviour. By the time of crop palpation at 0 h, the small amount of feed ingested before the disturbances may either have been too insignificant to be detected or it may already have passed on in the gastrointestinal system. The finding that more OF than HC chicks had water in the crop at 6 h, supports the idea that OF chicks did indeed ingest feed (although likely just a small amount) earlier than HC chicks, as water intake seems to occur later than feed intake ([de Jong et al., 2016](#)). The possible early intake of a small amount of feed may explain why body weight of the OF chicks was higher during the first week; utilisation of the yolk sac may be improved by early feeding ([van der Wagt et al., 2020](#)), i.e., early feeding, even small amounts, may stimulate the development of the gastrointestinal system. In a study of hatching on-farm where no handling of OF chicks was involved, [de Jong et al. \(2019\)](#) found that 41.5 % of the OF chicks had a crop containing feed upon arrival of HC chicks. However, this was determined by post-mortem examination of sacrificed chicks where even small crumbs could be identified. Furthermore, the deprivation period of HC chicks may have been longer than in the present study, allowing more time for the OF chicks to feed. Importantly, the hybrid used by [de Jong et al. \(2019\)](#) was the fast-growing Ross 308 which, due to genetic selection for fast growth, has an increased appetite ([Denbow, 1989](#); [Siegel and Wolford, 2003](#)).

Secondly, it appears that the need for resting after hatching (e.g. to recover from breaking out of the eggshell and drying) is better accommodated by the hatching on-farm condition, as even in fast-growing broilers hatching on-farm, where no handling is involved, OF chicks spend considerably more time resting than HC chicks on D0 ( $60.8 \pm 5.3$  % v.  $12.1 \pm 4.5$  %; [de Jong et al., 2016](#)). Staying in the hatching environment reduces the number of stressful events during the early post-hatch period and is likely to provide comfort to the chicks, accommodating more resting. Consistent with this, OF chicks were observed to be resting more during the first 23 h and more likely to have an empty crop at 6 h, 12 h and 36 h. However, despite the higher proportions of empty crops at 12 and 36 h, more OF than HC chicks were observed feeding during the observation periods starting 11 h and 35 h after arrival of HC chicks, but this may be due to OF chicks lagging behind in terms of feed intake due to more resting in the hours before. Thus, despite having the opportunity to feed and drink straight after hatching, results on crop content and behaviour indicate that OF chicks initially did not fully exploit the opportunity, as they seemed to

prioritise resting instead. As this prioritisation did not compromise growth, the increased resting in OF chicks compared to HC chicks may be beneficial to welfare, as rest assists in helping animals cope with stress and adapt to the environment ([Blokhuis, 1984](#); [Malleau et al., 2007](#)).

Hatching on-farm had long-lasting effects on fearfulness both in relation to humans (SPT) and novelty (NOT), the latter being a measure of more general fear, which seems unrelated to fear of humans ([Graml et al., 2008](#)). Both general fearfulness and the human-animal relationship are considered important aspects of animal welfare. When differences between treatments were found in the present study, OF chickens were less fearful than HC chickens. In SPT, the reduced fearfulness lasted for the first week (D1 and D7), whereas the tendency for reduced fearfulness in OF found in NOT was a treatment effect, i.e., across all ages. It has previously been shown that rearing chicks in settings simulating more natural conditions reduces fearfulness. For example, layer chicks brooded by either mother hens or brooders are less fearful than non-brooded chicks, both in fear tests and as indicated by behaviour in their home environments, and the effects have been shown to last at least four weeks and in some cases much longer ([Rodén and Wechsler, 1998](#); [Perre et al., 2002](#); [Rodenburg et al., 2009](#); [Shimmura et al., 2010](#); [Riber and Guzman, 2016](#)). Thus, providing what is likely perceived as a safe environment in the early life of chicks seems to reduce fearfulness. The possibility of remaining in the hatching environment, introduced by hatching on-farm, whereby a number of stressful events in early life are excluded, may increase the chickens' perception of the environment being safe. In support of this, [Giersberg et al. \(2020\)](#) found that fast-growing OF chicks vocalised less than HC chicks when placed in a novel environment on D1, which could be interpreted as decreased motivation for social reinstatement and better coping with the stressful event of being isolated in a novel environment. Furthermore, [Hedlund et al. \(2019\)](#) found unhandled layer chicks to be less fearful compared to chicks subjected to hatchery procedures on the day after exposure. However, in both studies the effect had vanished by the second testing (D21 and D36, respectively). Moreover, in the study by [Giersberg et al. \(2020\)](#), the OF chickens were found to be more fearful than HC chickens in two other fear tests (conducted from 8 to 23 days of age), thus contradicting the results in the present study. This seems peculiar and warrants more investigations, but perhaps it may be explained by differences between the studies in chicken hybrids and housing conditions used as well as the level of handling that the chicks were subjected to during early life.

Unexpectedly, the reduced general fearfulness of OF chickens in the current study did not result in increased use of the veranda or range. It has been suggested that general fearfulness is negatively correlated with the use of a range in slower-growing broilers ([Stadig et al., 2017](#)). This was partly confirmed in a study of fast-growing broilers with range access ([Taylor et al., 2020](#)). In contrast, we found no differences between treatments in use of neither the veranda nor the range, despite that OF chickens were found to be less fearful. A possible explanation may be that the reduced general fearfulness (measured in NOT) was only a tendency in one of the three measures, likely not sufficiently strong to affect a highly fear-eliciting stimuli such as a novel outdoor environment.

A point to take into consideration in the discussion of the effects of on-farm hatching on animal welfare is the immediate post-hatch deprivation period, which depends on the hatching window, the time needed for the hatchery procedures, the duration of transportation time and time spent on unloading and placing the HC chicks. Previous studies have shown that the longer the duration of the deprivation period, the more likely it will affect mortality and growth of the chickens and the longer-lasting the negative effects will be ([Gonzales et al., 2003](#); [de Jong et al., 2017](#)). In the present study, the deprivation period was rather short, being maximum 25 h for the early hatchers and down to only 5 h for the late hatchers. This is nearly within the maximum post-hatch deprivation period of 24 h suggested to be the limit at which broiler

productivity at slaughter age is preserved (Gonzales et al., 2003). Thus, it is reasonable to assume that greater effects of hatching location would have been observed on several of the welfare indicators if the deprivation period had been longer. Furthermore, the study took place during an exceptionally wet autumn and winter period, and we speculate whether results on use of veranda and outdoor area would have been different had the experiment been conducted during the summer period.

## 5. Conclusion

On-farm hatching of slower-growing broilers appears to be a benefit to animal welfare. Particularly, when considering that these positive results were achieved even though the study included handling of the OF chicks and that HC chicks were only feed and water deprived for a relatively short period during early life. However, due to the limited knowledge and the number of influencing factors (e.g. duration of deprivation, amount of handling, on-farm hatching system, broiler hybrid and general housing conditions), more research should be addressed to the welfare consequences of hatching on-farm, particularly to effects on fearfulness.

## Declaration of Competing Interest

The authors report no declarations of interest.

## Acknowledgements

We are grateful to Estelle Leroux, Agrocampus Ouest, France, for assisting with data collection and data management during her internship. We thank post doc Fernanda M. Tahamtani for assisting with data collection and Dr. Ingrid de Jong, Wageningen Livestock Research, the Netherlands, for valuable feedback on an earlier version of this manuscript. A special thanks to all the different segments of the broiler production chain – without their willingness to participate we could not have conducted this study. The research described in this paper has been commissioned and funded by the Ministry of Environment and Food of Denmark as part of the “Contract between Aarhus University and Ministry of Environment and Food for the provision of research-based policy advice at Aarhus University, 2017-2020”.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.applanim.2021.105319>.

## References

- Aitchison, J., 1955. On the distribution of a positive random variable having a discrete probability mass at the origin. *J. Am. Stat. Assoc.* 50, 901–908.
- Archer, G.S., Mench, J.A., 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.
- Bergoug, H., Guinebretiere, M., Tong, Q., Roulston, N., Romanini, C.E., Exadaktylos, V., Berckmans, D., Garain, P., Demmers, T.G., McGonnell, I.M., Bahr, C., Burel, C., Eterradossi, N., Michel, V., 2013. Effect of transportation duration of 1-day-old chicks on postplacement production performances and pododermatitis of broilers up to slaughter age. *Poult. Sci.* 92, 3300–3309.
- Blokhuis, H.J., 1984. Rest in poultry. *Appl. Anim. Behav. Sci.* 12, 289–303.
- Boissy, A., 1995. Fear and fearfulness in animals. *Q. Rev. Biol.* 70, 165–191.
- Brantsæter, M., Tahamtani, F.M., Nordgreen, J., Sandberg, E., Hansen, T.B., Rodenburg, T.B., Moe, R.O., Janczak, A.M., 2017. Access to litter during rearing and environmental enrichment during production reduce fearfulness in adult laying hens. *Appl. Anim. Behav. Sci.* 189, 49–56.
- Careghi, C., Tona, K., Onagbesan, O., Buyse, J., Decuyper, 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, 1314–1320.
- Dawkins, M.S., Cook, P.A., Whittingham, M.J., Mansell, K.A., Harper, A.E., 2003. What makes free-range broiler chickens range? In situ measurement of habitat preference. *Anim. Behav.* 66, 151–160.
- de Jong, I., van Riel, J., Lourens, S., Bracke, M., van den Brand, H., 2016. Effects of Food and Water Deprivation in Newly Hatched Chickens: A Systematic Literature Review and Meta-Analysis. Wageningen Livestock Research, Wageningen, The Netherlands, p. 70.
- de Jong, I.C., van Riel, J., Bracke, M.B.M., van den Brand, H., 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., 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, 1269–1277.
- 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, 4662–4671.
- Denbow, D.M., 1989. Peripheral and central control of food intake. *Poult. Sci.* 68, 938–947.
- EC, 2007. Council Regulation (EC) No 834/2007 of 28 June 2007 on Organic Production and Labelling of Organic Products and Repealing Regulation (EEC) No 2092/91. <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02007R0834-20130701>.
- EFSA, 2011. Scientific opinion concerning the welfare of animals during transport. *EFSA J.* 9, 1966.
- Fletcher, D., 2008. Confidence intervals for the mean of the delta-lognormal distribution. *Environ. Ecol. Stat.* 15, 175–189. <https://doi.org/10.1007/s10651-007-0046-8>.
- Fraser, D., 2008. Understanding animal welfare. *Acta Vet. Scand.* 50 (Suppl. 1), S1.
- Giersberg, M.F., Poolen, I., de Baere, K., Gunnink, H., van Hattum, T., van Riel, J.W., de Jong, I.C., 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.
- Gonzales, E., Kondo, N., Saldanha, E.S., Loddy, M.M., Careghi, C., Decuyper, E., 2003. Performance and physiological parameters of broiler chickens subjected to fasting on the neonatal period. *Poult. Sci.* 82, 1250–1256.
- Graml, C., Waiblinger, S., Niebuhr, K., 2008. Validation of tests for on-farm assessment of the hen-human relationship in non-cage systems. *Appl. Anim. Behav. Sci.* 111, 301–310.
- 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, 2367.
- Henriksen, S., Bilde, T., Riber, A.B., 2016. Effects of post-hatch brooding temperature on broiler behavior, welfare, and growth. *Poult. Sci.* 95, 2235–2243.
- Hollems, 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, 2534–2542.
- Hubbard, n.d. Hubbard Premium - growth rates of intermediate and conventional broiler chickens. [https://www.hubbardbreeders.com/media/web\\_leaflet\\_hubbard\\_premium\\_intermed\\_conv\\_dutch.pdf](https://www.hubbardbreeders.com/media/web_leaflet_hubbard_premium_intermed_conv_dutch.pdf) (accessed 31 January 2021).
- Jacobs, L., Delezie, E., Duchateau, L., Goethals, K., Ampe, B., Lambrecht, E., Gellynck, X., Tuytens, F.A., 2016. Effect of post-hatch transportation duration and parental age on broiler chicken quality, welfare, and productivity. *Poult. Sci.* 95, 1973–1979.
- Jacobs, L., Delezie, E., Duchateau, L., Goethals, K., Ampe, B., Buyse, J., Tuytens, F.A.M., 2017. Impact of transportation duration on stress responses in day-old chicks from young and old breeders. *Res. Vet. Sci.* 112, 172–176.
- Landbruksstyrelsen, 2020. Vejledning for Økologisk Jordbrug [In Danish: Guidelines for Organic Farming]. [https://lbst.dk/fileadmin/user\\_upload/NaturErhverv/Filer/Indsatsmaeraeder/Oekologi/Jordbrugsbedrifter/Vejledning\\_til\\_oekologisk\\_jordbrugsproduktion/OEokologivejledning\\_februar2020.pdf](https://lbst.dk/fileadmin/user_upload/NaturErhverv/Filer/Indsatsmaeraeder/Oekologi/Jordbrugsbedrifter/Vejledning_til_oekologisk_jordbrugsproduktion/OEokologivejledning_februar2020.pdf).
- Malleau, A.E., Duncan, L.J.H., Widowski, T.M., Atkinson, J.L., 2007. The importance of rest in young domestic fowl. *Appl. Anim. Behav. Sci.* 106, 52–69.
- Mancinelli, A.C., Mugnai, C., Castellini, C., Mattioli, S., Moscati, L., Piottoli, L., Amato, M.G., Doretti, M., Dal Bosco, A., Cordovani, E., Abbate, Y., Ranucci, D., 2018. Effect of transport length and genotype on tonic immobility, blood parameters and carcass contamination of free-range reared chickens. *Italian J Anim. Sci.* 17, 557–564.
- Mitchell, M.A., 2009. Chick transport and welfare. *Avian Biol. Res.* 2, 99–105.
- One2Born, n.d. The innovative hatchholder One2Born, <http://www.one2born.com/en/> (accessed 31 January 2021).
- Perre, Y., Wauters, A.M., Richard-Yris, M.A., 2002. Influence of mothering on emotional and social reactivity of domestic pullets. *Appl. Anim. Behav. Sci.* 75, 133–146.
- Pinchasov, Y., Noy, Y., 1993. Comparison of post-hatch holding time and subsequent early performance of broiler chicks and turkey poults. *Br. Poult. Sci.* 34, 111–120.
- R Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Riber, A.B., Guzman, D.A., 2016. Effects of dark brooders on behavior and fearfulness in layers. *Animals* 6, 3.
- Roden, C., Wechsler, B., 1998. A comparison of the behaviour of domestic chicks reared with or without a hen in enriched pens. *Appl. Anim. Behav. Sci.* 55, 317–326.
- Rodenburg, T.B., Uitdehaag, K.A., Ellen, E.D., Komen, J., 2009. The effects of selection on low mortality and brooding by a mother hen on open-field response, feather pecking and cannibalism in laying hens. *Anim. Welf.* 18, 427–432.
- Shimmura, T., Kamimura, E., Azuma, T., Kansaku, N., Uetake, K., Tanaka, T., 2010. Effect of broody hens on behaviour of chicks. *Appl. Anim. Behav. Sci.* 126, 125–133.
- Shira, E.B., Sklan, D., Friedman, A., 2005. Impaired immune responses in broiler hatchling hindgut following delayed access to feed. *Vet. Immunol. Immunopathol.* 105, 33–45.
- Siegel, P.B., Wolford, J.H., 2003. A review of some results of selection for juvenile body weight in chickens. *J. Poult. Sci.* 40, 81–91.

- Simon, K., de Vries Reilingh, G., Bolhuis, J.E., Kemp, B., Lammers, A., 2015. Early feeding and early life housing conditions influence the response towards a noninfectious lung challenge in broilers. *Poult. Sci.* 94, 2041–2048.
- Souza da Silva, C., Molenaar, R., Giersberg, M.F., Rodenburg, T.B., van Riel, J.W., De Baere, K., Van Dosselaer, I., Kemp, B., van den Brand, H., de Jong, I.C., 2021. Day-old chicken quality and performance of broiler chickens from 3 different hatching systems. *Poult. Sci.* 100, 100953.
- Stadig, L.M., Rodenburg, T.B., Ampe, B., Reubens, B., Tuytens, F.A.M., 2017. Effect of free-range access, shelter type and weather conditions on free-range use and welfare of slow-growing broiler chickens. *Appl. Anim. Behav. Sci.* 192, 15–23.
- Taylor, P.S., Hensworth, P.H., Groves, P.J., Gebhardt-Henrich, S.G., Rault, J.L., 2020. Frequent range visits further from the shed relate positively to free-range broiler chicken welfare. *Animal* 14, 138–149.
- Uni, Z., Ferket, R.P., 2019. Methods for early nutrition and their potential. *Worlds Poult. Sci. J.* 60, 101–111.
- 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, 58–67.
- van de Ven, L.J., van Wagenberg, A.V., Groot Koerkamp, P.W., Kemp, B., van den Brand, H., 2009. Effects of a combined hatching and brooding system on hatchability, chick weight, and mortality in broilers. *Poult. Sci.* 88, 2273–2279.
- van der Wagt, I., de Jong, I.C., Mitchell, M.A., Molenaar, R., van den Brand, H., 2020. A review on yolk sac utilization in poultry. *Poult. Sci.* 99, 2162–2175.
- Waiblinger, S., Boivin, X., Pedersen, V., Tosi, M.-V., Janczak, A.M., Visser, E.K., Jones, R. B., 2006. Assessing the human–animal relationship in farmed species: a critical review. *Appl. Anim. Behav. Sci.* 101, 185–242.
- Willemsen, H., Debonne, M., Swennen, Q., Everaert, N., Careghi, C., Han, H., Bruggeman, V., Tona, K., Decuyper, E., 2010. Delay in feed access and spread of hatch: importance of early nutrition. *Worlds Poult. Sci. J.* 66, 177–188.