



Comparative assessment of general behaviour and fear-related responses in hatchery-hatched and on-farm hatched broiler chickens

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ABSTRACT

Traditionally, broiler chickens hatch in the hatchery and they are usually not provided with feed and water until placement at the farm. This can have negative effects on their health and welfare. Therefore, alternative systems providing early nutrition, for instance by hatching eggs in a poultry house (on-farm hatching) are increasingly being used in practice. However, information on the behaviour and welfare of on-farm hatched chickens in relation to hatchery-hatched chickens is very limited. This study aims to gain basic knowledge of the behaviour of on-farm hatched chickens (OH) by comparing them to a control group (C) hatched in the hatchery. In addition, fear-related responses were assessed as indicators of chicken welfare. About 13,800 chickens per treatment group were reared in three consecutive batches in eight floor pens under semi-commercial conditions. Direct behavioural observations and three different fear tests, i.e. a novel environment, a human approach and a novel object test, were carried out between two and 36 days of age. Except for 'disturbance behaviour' (i.e. pushing or overrunning another chicken), which was more often performed by the OH chickens ($F_{1,3} = 35.10$, $P < 0.05$), no effect of treatment was found on general behaviour. In contrast, nearly all observed behaviours were affected by the chickens' age ($F_{4,24} = 4.02-41.81$, $P < 0.05$). In the fear tests, most variables, for instance average latency of chickens touching a human and the number of chickens in the vicinity of a novel object, differed between the treatments ($P < 0.05$) with OH chickens being more fearful and less active. The present results indicate that the hatching system (hatchery-hatching vs. on-farm hatching) seems to have limited effects on broiler chicken activity and general behaviours. In test situations, however, hatchery-hatched chickens showed more active and less fearful responses compared to on-farm hatched chickens. The underlying causes for these differences in response to more challenging situations remain to be investigated further, as these may be related to a higher intrinsic motivation to search for food or more exposure to humans or objects in the hatchery in C chickens as compared to OH chickens, but also to differences in coping style or development of cognitive abilities between the treatment groups.

1. Introduction

In commercial hatcheries, the majority of broiler chickens hatch in conventional hatchers without access to feed and water until placement at the farm. Day-old chickens are able to survive without exogenous food and water by utilizing energy reserves from their yolk sac within the first 72 h of life (Mitchell, 2009; EFSA, 2011). According to European legislation chickens must therefore not be deprived of nutrition for

longer than 72 h after hatch (Council Regulation 1/2005/EC, 2005). Chickens from the same batch usually do not hatch at exactly the same time but within a hatch window of about 24–48 h (Careghi et al., 2005), depending on breeder flock characteristics and incubation conditions (Lourens et al., 2005). As a result, some of the birds are likely up to two days old when pulled from the hatcher. The fastening period of the chickens is further increased by several processing procedures at the hatchery (e.g. sorting and vaccination), a storage time of 1–4 h before

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transport, and duration of transport to and unloading at the farm (Hollemans et al., 2018).

Delayed access to nutrition has been shown to negatively affect post-hatch growth performance (Bigot et al., 2003), particularly in fast growing broiler chicken strains (Gonzales et al., 2008). In addition, delayed growth of organs involved in the immune response and a reduced lymphocyte proliferation were found with post-hatch feed deprivation (Panda et al., 2015). This can result in an increased susceptibility to disease in case of a high antigenic pressure (Simon et al., 2015) but this requires further study (De Jong et al., 2017). Although single studies did not find consistent effects of early feed deprivation on mortality, a recent meta-analysis by De Jong et al. (2017) showed that fasting periods of on average 48 h resulted in higher mortality rates at six weeks of age compared to 0 and 24 h of fasting. Thus, a prolonged delay of first feed and water intake seems to have negative effects on performance, health and welfare, which are likely exacerbated for those chickens that hatch early within a given hatch window (Van de Ven et al., 2011; Lamot et al., 2014). As a consequence, a Dutch court recently decided that, within a five-year transition period, all chicks in the Netherlands must receive feed and water within 36 h after hatching of the first chick in the batch (Uitspraak ECLI:NL:CBB:2018:309, 2018).

In practice, early nutrition can be supplied in special hatching systems in the hatchery (Van der Pol et al., 2015). Another option is to hatch eggs on-farm. Various on-farm hatching systems exist, varying in lay-out and degree of automation (De Jong et al., 2019). A common characteristic of these systems is that eggs are transported to and placed in the broiler house at day 18 of incubation. After hatch, the chickens immediately have access to feed and water provided in the house. Although several studies have investigated single factors associated with these systems, for instance the absence of transport of day-old chicks (Hollemans et al., 2018), limited system comparisons between on-farm and conventional hatching in the hatchery have been performed on the welfare, health and performance of broiler chickens in these systems. De Jong et al. (2019) compared on-farm hatched broiler chickens with conventional hatched flocks reared on the same commercial farms. They found no differences in performance, measured as body weight, feed conversion ratio and mortality. However, on-farm hatched broilers had a lower prevalence of footpad dermatitis, which was interpreted to indicate improved welfare (De Jong et al., 2019) but other components of welfare, such as psychological well-being, which is most reliably indicated by the chickens' behaviour (Dawkins, 1999), were not assessed in that study.

Observing the occurrence and frequency of general behaviours, such as locomotion and resting patterns, may provide valuable information about the animals' time budgets in given environmental and social contexts but the implications for animal welfare of such behavioural scan-sampling measurements are not always clear. By contrast, behavioural responses to challenges such as avoiding a potentially aversive situation or working to gain access to a resource, may be more indicative of a bird's psychological well-being (Dawkins, 1999). Behaviours that fit in this concept are fear-related responses. In captive environments, excessive fear of humans or management procedures can lead to chronic stress, and thus impair animal welfare. In poultry, fear responses can be assessed by means of a set of several validated tests (Forkman et al., 2007), which are feasible in commercial and semi-commercial environments. Some of these tests, for instance the human approach test, can be carried out at a group level in the chickens' home pen, which makes them less disturbing for the animals.

The aim of the present study was to compare general behaviours and several fear-related responses of conventional chickens (C) hatched in a hatchery with on-farm hatched chickens (OH) from day-old until slaughter age. It was hypothesized that C chickens would show more active behaviours and responses in early life due to a higher motivation to search for feed and water. Furthermore, it was expected that the hatching environment could alter behavioural responsiveness later in life, as stress at an early age, caused by e.g. handling in the hatchery and

transport, may have long-term consequences on behaviour and fear responses (Hedlund et al., 2019; Ericsson et al., 2016).

2. Material and methods

2.1. Experimental design and housing

The study was carried out at the Experimental Poultry Centre in Geel, Belgium. A total of 27,600 Ross 308 broiler chickens was reared in three consecutive batches from August 2016 to January 2017. Four separate compartments of a broiler house that were accessible from a central hallway were used. Each compartment consisted of two adjacent pens (each measuring 6 × 9.4 m) which were separated by a wire mesh covered with a hardboard plate to prevent bird-to-bird contact. Each pen was connected to a central heating, and was equipped with its own automated feeder pans and nipple drinker lines. Chopped wheat straw, first pelleted and then crumbled, served as litter material (1.5 kg/m²). In each compartment, a treatment pen in which the chickens hatched on-farm (OH) was paired with a control pen (C) in which conventional hatchery-hatched chickens were housed. In the OH pens, the X-treck system (Vencomatic, Eersel, The Netherlands) was installed. This system consists basically of a metal frame for setter trays with eggs mounted above a polypropylene belt (33 cm above the ground). After hatching in the trays, the chickens fall on the belt (which is covered with a thin layer of crumbled straw pellets) where they dry and from which they can reach the floor of the pen, and thus feed and water. Trays with egg shells and non-hatched eggs are removed from the barn and the metal frame is lifted to the ceiling after hatching. The location of the C and the OH pen relative to the central door was alternated per compartment but was the same in all batches. This setup resulted in four replicates per treatment per batch, which were repeated in three consecutive batches. Data from this experiment on performance and animal health are presented and discussed elsewhere (De Jong et al., 2020).

2.2. Animals and management procedures

C and OH chickens (as hatched) in the same batch originated from the same batch of eggs of a Ross 308 breeder flock, aged between 35 and 41 weeks. All eggs were incubated for 18 days at a commercial hatchery (Spoormans, Arendonk, Belgium). At day 18 of incubation, trays were alternately assigned to either the C or the OH treatment by the hatchery. C eggs remained in the hatchery where they were further incubated in hatching baskets until the majority of the chickens had hatched (21 days of incubation). The chickens were then pulled from the incubator and subjected to standard commercial procedures, such as selection of second grade chickens. C chickens were transported to the farm at day (d) 0, whereas OH eggs were transported to the farm at d18 of incubation and placed in the X-treck system. In both cases, the transport time was approximately 45 min. In batch 1, 2 and 3, 1207, 1185 and 1185 eggs were placed on the setter trays in each OH pen respectively. Almost all OH chicks had hatched by the end of day 20 of incubation, and in the morning of the arrival of the C chickens, the caretakers of the farm removed all non-hatched eggs and second grade chickens from the OH pens. This resulted in 1176, 1165 and 1154 chickens per OH pen at d0 in batch 1, 2 and 3 respectively. The number of eggs placed in the second and third batch was adjusted according to the hatching performance in the previous batches (for details see De Jong et al., 2020). In C pens, 1150 day-old chickens were placed at d0. Feed was available on chick paper during the first days, starting at d -3 in the OH pens, and followed a commercial four phase ad libitum feeding program thereafter. From d -3 to d0, continuous light was provided to enable OH chickens to find feed and water immediately. The light regime started with 23L:1D (d0) and was gradually decreased until 18L:6D at d6. Three days before depopulation, the light regime was increased to 23L:1D. Light intensity was 20 LUX at animal height in all pens. Ambient temperature decreased from 35 °C (d0) to 19 °C (d40). In the OH pens, the ambient temperature

between d -3 and d0 was adjusted based on the recorded egg shell temperatures (target value: 37.8 °C). The room temperature was 35 °C on average (for details see De Jong et al., 2020). At d33, thinning was performed as a standard management procedure with 280 chickens being removed from each pen. The remaining chickens stayed on the farm until depopulation at 40 days of age. All chickens were vaccinated in the broiler house against Infectious Bronchitis and Newcastle Disease at d0 and 13, and against Gumboro at d19. Antimicrobial or other veterinary treatment was not necessary during the entire experiment.

2.3. Behavioural observations and tests

Direct behavioural observations were carried out between two and 36 days of age. In addition, the animals were subjected to three behavioural tests assessing fear responses: novel environment test, novel object test and human approach test. Observation and testing order on each study day were randomized at pen level. Since the X-treck system was present in OH pens, the observers were not blinded to treatment. An overview of the behavioural observations and tests performed at different ages in the three batches of broiler chickens is provided in Table 1. Three experimenters were trained beforehand and conducted all of the behavioural observations and the behavioural tests, of which the first tests were always performed together to ensure that procedures were carried out in the same way by the observers.

2.3.1. Behavioural observations

Direct behavioural observations based on a scan sampling method were carried out between 2 and 36 days of age between 9:00 and 16:00 h. Each pen was virtually divided by a grid with five columns and ten rows. For the observations, three areas within the grid were randomly chosen per pen. The experimenter walked about 1.5 m away from the observation area and squatted for a habituation period of two minutes. Thereafter, the number of birds performing each of eleven predefined behaviours was recorded. The list of major behavioural states contained: sitting/lying, standing, walking, preening (while standing or sitting), dustbathing (all elements as described by Van Liere, 1991), eating, drinking, floor pecking (while standing or sitting), feather pecking (gentle and severe), disturbance (i.e. pushing or overrunning another chicken, so that the disturbed chicken stops its current activity), and aggression (all elements as described by Ventura et al., 2012). The scans were repeated four times per area and then the next area in a pen was observed; after observing the three areas per pen the observer moved to the next pen. In addition, the variable 'total active' summarized all of the behaviours standing, walking, preening, dustbathing, floor pecking, feather pecking and aggression. For further analyses, the percentages of birds assigned to each of the states was calculated.

Table 1

Overview of behavioural observations and tests performed during three consecutive batches of broiler chickens (1-3) and at specific ages in days (d). Due to slightly different testing days per batch, ages were summarized for further analysis where necessary.

| Parameter | Batch | Animal age (d) | Age summarized |
|---------------------------------|-------|------------------|--|
| Direct behavioural observations | 1 | 2, 5, 12, 20 | |
| | 2 | 2, 5, 12, 20, 36 | – |
| | 3 | 5, 12, 20, 36 | |
| Novel environment (NE) test | 1 | 1, 8 | |
| | 2 | 1, 8 | – |
| | 3 | 1 | |
| Novel object (NO) test | 1 | 16, 23 | 2 nd , 3 rd week of life |
| | 2 | 9, 16 | 1 st , 2 nd week of life |
| | 3 | 8, 14, 21 | 1 st , 2 nd , 3 rd week of life |
| Human approach (HA) test | 1 | 16, 23 | 2 nd , 3 rd week of life |
| | 2 | 9, 16 | 1 st , 2 nd week of life |
| | 3 | 8, 14, 21 | 1 st , 2 nd , 3 rd week of life |

2.3.2. Behavioural tests

2.3.2.1. Novel environment test. A novel environment (NE) test based on the protocol by de Haas et al. (2014) was conducted at 1 and 8 days of age. On each of these days, two birds per location in a pen (i.e. near the feeders, the drinkers and the wall) were caught and tested one-by-one in the central hall (n = 24 animals/treatment per batch). A non-transparent black round bucket (23.5 cm in diameter at the bottom, 23.5 cm height) served as NE. After placing a bird in the bucket, its individual response was recorded for two minutes. The experimenter documented latency to vocalize, number of vocalizations, latency of the first flight attempt, and number of flight attempts while standing out of sight of the bird.

2.3.2.2. Human approach test. A human approach (HA) test was carried out in the first three weeks of age (Table 1). The observer walked to one of the testing locations (i.e. near the feeders, the drinkers and the wall) in each pen and stood there for a total of three minutes. During the test, the number of chickens within a semi-circle of one meter in front of the experimenter was counted every 30 s. The latency of the first chicken to touch the boots of the observer was also recorded. The test was repeated on the three different locations in each pen before testing the next pen.

2.3.2.3. Novel object test. A novel object (NO) test was performed on the same locations and at the same ages (d1 and 8) as the human approach test. The NO was a golf ball covered with aluminium foil. After it was placed on the floor, the observer walked about 3 m away from the object. The chickens were observed for three minutes during which the latency of the first bird to approach (< 25 cm) and the latency of the first bird to touch the object were recorded. In addition, the observer counted the number of chickens within a 25 cm radius of the novel object every 30 s. The test was repeated on the three different locations in each pen before testing the next pen.

2.4. Statistical analyses

All analyses were performed with the program GenStat (version 19.1, VSN International). Since treatments were allocated to individual pens which were similar for the three batches, the scores of individual chickens were aggregated (over batch) per pen per age. The normality of the data was checked using residual plots. A natural log transformation of the aggregated measure was applied when its variance increased for increased levels of the measure. A pen within a room was the experimental unit for the main effect of treatment, and non-significant block effects for room were excluded in the final model. Proportions of chickens performing the different general behaviours and the responses measured in the NE, HA and NO tests (except for the repeated counts at 6 different points in time in the NO and HA tests) were analysed using a split plot model using ANOVA, with age within pen as residual term. In these split plot models the effect of age and the interaction of treatment by age was tested against the residual variation. The repeated counts in the NO and HA tests were analysed using a mixed model in which time point within age and pen was added as an extra stratum in the split plot model, and different variances between pens were simultaneously estimated per age. The fixed effect of time was estimated as a linear effect. P-values < 0.05 were considered to be statistically significant, P-values between 0.05 and 0.10 were considered to indicate a trend. All data are presented as (back transformed) mean ± standard error of the mean (SEM) of the aggregated data (over batch) per pen per age.

2.5. Ethical note

The study was carried out in compliance with the ethical guidelines of the International Society of Applied Ethology (Sherwin et al., 2003). All birds were housed according to EU law (Council Directive 2007/43/EC, 2007). The experiments were approved by the Central

Commission of Animal Experiments (approval no: AVD10300201563) and the Institutional Animal Care and Use Committee.

3. Results

3.1. Behavioural observations

The proportion of C and OH birds performing the different behaviours as well as the effects of treatment, age and the interaction between treatment and age are summarized in Table 2. An effect of treatment was only found for the variable 'disturbance' ($F_{1,3} = 35.10$, $P < 0.05$), with OH chickens disturbing their pen mates more often compared to C chickens. There was a significant treatment x age interaction for the proportion of birds eating ($F_{4,24} = 3.37$, $P < 0.05$), and a tendency for such an interaction for sitting/lying behaviour ($F_{4,24} = 2.69$, $P = 0.06$). One peak of eating behaviour was observed in C birds at 12 days of age, which decreased thereafter. In contrast, OH chickens showed two peaks of eating behaviour, namely at 5 and 20 days. At the beginning of rearing (2 days of age), a higher proportion of C birds was eating compared to OH birds, whereas at the end (36 days) more OH birds were engaged in this behaviour. Following the peaks in eating behaviour, sitting/lying was lowest at 12 days of age in C birds, whereas OH groups showed the lowest proportions of birds sitting/lying at 5 and at 20 days. Age had an effect on nearly all of the observed behaviours ($F_{4,24} = 4.02$ – 41.81 , $P < 0.05$ - < 0.001), except for preening and drinking. For behaviours that require a certain level of activity, for instance dustbathing, eating and aggression, a peak was found at 12–20 days of age. Regarding floor pecking, a peak occurred already at 5 days. Correspondingly, higher proportions of birds were lying down in the beginning (2 days of age) and at the end of the production cycle (36 days of age). In contrast, the proportion of birds walking decreased continuously with age, whereas the opposite was observed for feather pecking.

3.2. Behavioural tests

3.2.1. Novel environment (NE) test

The results of NE test are presented in Fig. 1. Treatment tended to affect the number of flight attempts ($F_{1,6} = 5.59$, $P = 0.06$), with C chickens showing more flight attempts at both ages, respectively. For

vocalization frequency, a significant treatment x age interaction was found ($F_{1,6} = 7.72$, $P < 0.05$), with C chickens vocalizing more at d1 and less at d8 compared to OH chickens. Age affected the response variables latency to vocalize ($F_{1,6} = 9.57$, $P < 0.05$) and number of flight attempts ($F_{1,6} = 6.57$, $P < 0.05$). The chickens vocalized sooner and showed more flight attempts at d8 compared to d1. A tendency for an age effect was found for latency to the first flight attempt ($F_{1,6} = 5.51$, $P = 0.06$), with older chickens trying to escape sooner.

3.2.2. Human approach (HA) test

Treatment affected the average number of chickens approaching the human in the HA test (Wald statistic = 11.80, $P < 0.05$), and tended to affect the latency of the first chicken touching the human (Wald statistic = 10.14, $P = 0.05$). More C chickens approached the human (Fig. 2) and C chickens touched him sooner compared to the OH chickens (Fig. 3). Age had an effect on both response variables (number of chickens approached: Wald statistic = 162.79, $P < 0.001$; latency to touch human: Wald statistic = 63.77, $P < 0.001$). The highest average number of birds approached the human at 3 weeks of age, followed by 2 weeks and 1 week, whereas latency to touch the human decreased with increasing age (1 week of age < 2 weeks < 3 weeks). However, there was a significant interaction between treatment and age for the number of chickens approaching the human (Wald statistic = 10.52, $P < 0.01$), as the largest difference between C and OH was found in week 2, followed by week 3 and week 1. In addition, there was a significant interaction between age and time of the repeated counts (Wald statistic = 12.97, $P < 0.01$). In week 1, the number of chickens within 1 m of the human was more or less similar during the test, whereas in weeks 2 and 3 the number of chickens increased with time (Fig. 2).

3.2.3. Novel object (NO) test

Treatment effects were found for the average number of chickens within a 25 cm radius of the novel object in the NO test (Wald statistic = 31.84, $P < 0.001$) and for latency of the first bird to approach (Wald statistic = 16.94, $P < 0.001$). A higher number of C chickens was observed near the NO (Fig. 4), and they approached it sooner compared to OH chickens (Fig. 5A). There was a tendency of C chickens touching the NO earlier than OH chickens (Wald statistic = 4.0, $P = 0.07$; Fig. 5B). The number of chickens within the 25 cm radius of the NO was further

Table 2

Proportions of control (C) and on-farm hatched (OH) chickens performing distinct behaviours at different ages.

| Behaviour | Treatment | Age (days) | | | | | $P_{\text{treatment}}$ | P_{age} | $P_{\text{treatment} \times \text{age}}$ |
|-----------------|-----------|------------|-------|-------|-------|-------|------------------------|------------------|--|
| | | 2 | 5 | 12 | 20 | 36 | | | |
| Standing | C | 8.32 | 5.27 | 7.54 | 8.48 | 5.36 | ns | < 0.001 | ns |
| | OH | 8.78 | 6.19 | 7.32 | 8.58 | 6.67 | | | |
| Walking | C | 10.66 | 8.31 | 9.38 | 8.04 | 2.05 | ns | < 0.001 | ns |
| | OH | 10.29 | 8.61 | 8.15 | 6.97 | 2.79 | | | |
| Preening | C | 5.51 | 4.02 | 4.19 | 4.78 | 4.67 | ns | ns | ns |
| | OH | 4.64 | 4.70 | 4.82 | 4.54 | 3.80 | | | |
| Dustbathing | C | 0.00 | 0.22 | 0.85 | 0.14 | 0.55 | ns | < 0.001 | ns |
| | OH | 0.05 | 0.18 | 0.29 | 0.10 | 0.18 | | | |
| Floor pecking | C | 1.05 | 13.15 | 7.55 | 4.61 | 4.19 | ns | < 0.001 | ns |
| | OH | 2.86 | 11.68 | 6.84 | 4.21 | 2.75 | | | |
| Feather pecking | C | 0.00 | 0.09 | 0.08 | 0.15 | 0.24 | ns | < 0.001 | ns |
| | OH | 0.00 | 0.00 | 0.06 | 0.13 | 0.21 | | | |
| Aggression | C | 0.00 | 0.09 | 0.33 | 0.35 | 0.00 | ns | < 0.05 | ns |
| | OH | 0.00 | 0.06 | 0.23 | 0.44 | 0.13 | | | |
| Total active | C | 25.54 | 31.15 | 29.92 | 26.55 | 17.05 | ns | < 0.001 | ns |
| | OH | 26.61 | 31.41 | 27.72 | 24.96 | 16.53 | | | |
| Sitting/lying | C | 51.14 | 44.10 | 35.77 | 39.98 | 61.09 | ns | < 0.001 | 0.06 |
| | OH | 55.34 | 10.29 | 48.37 | 42.03 | 58.71 | | | |
| Disturbance | C | 1.27 | 1.08 | 1.51 | 2.08 | 2.24 | < 0.05 | < 0.001 | ns |
| | OH | 2.35 | 0.56 | 1.86 | 2.33 | 2.00 | | | |
| Eating | C | 11.20 | 16.39 | 25.27 | 23.76 | 13.01 | ns | < 0.001 | < 0.05 |
| | OH | 9.15 | 19.80 | 15.09 | 22.33 | 17.49 | | | |
| Drinking | C | 10.86 | 7.28 | 7.53 | 7.63 | 6.61 | ns | ns | ns |
| | OH | 6.54 | 7.93 | 6.96 | 8.35 | 5.28 | | | |

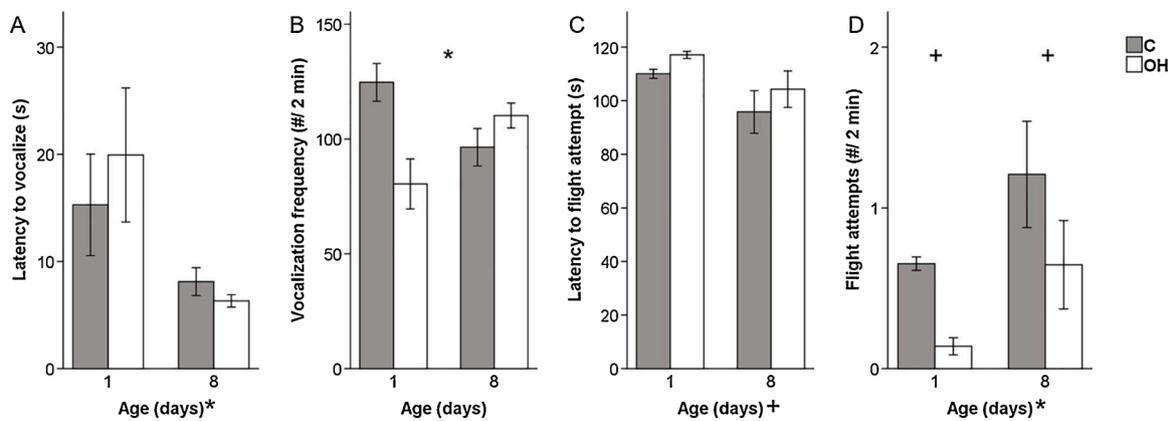


Fig. 1. Responses in the novel environment (NE) test at 1 and 8 days of age (n = 72 and 48 chickens/treatment respectively): A) Mean latency to vocalize (\pm SEM), B) mean vocalization frequency (\pm SEM), C) mean latency to flight attempt and D) mean number of flight attempts of control (C) and on-farm hatched (OH) chickens. *Between two pairs of bars denotes an interaction between age and treatment ($P < 0.05$). *After ‘Age (days)’ denotes an age effect ($P < 0.05$). +Between bars denotes a tendency of treatment ($P < 0.1$). +After ‘Age (days)’ denotes a tendency of age ($P < 0.1$).

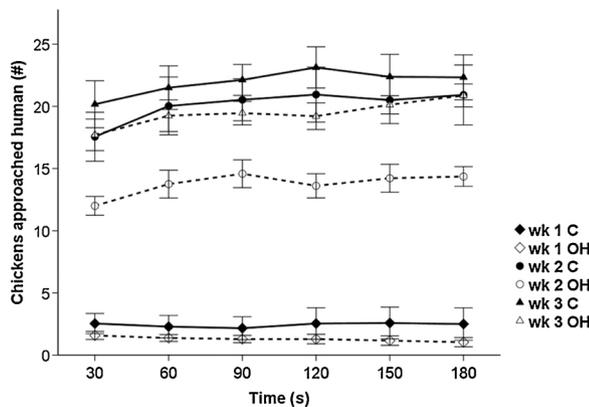


Fig. 2. Responses in the human approach (HA) test at different times during the test (30-180 s): Mean number (\pm SEM) of chickens approaching the human at 1, 2, and 3 weeks of age (wk) for control (C) and on-farm hatched chickens (OH). Significant effects were found for treatment, age, the interaction between treatment and age, and the interaction between age and time ($P < 0.05$).

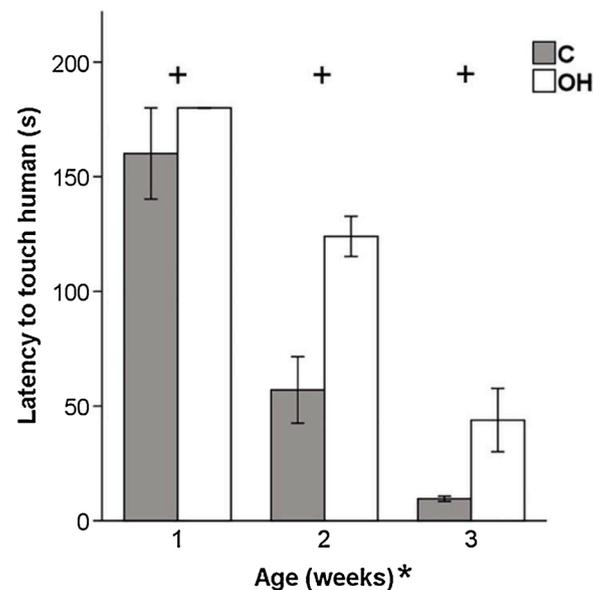


Fig. 3. Responses in the human approach (HA) test: Mean latency (\pm SEM) of chickens touching the human at 1, 2, and 3 weeks of age for control (C) and on-farm hatched chickens (OH). *After ‘Age (days)’ denotes an age effect ($P < 0.05$). +Between bars denotes a tendency of treatment ($P < 0.1$).

affected by the interaction between age and time point of observation during the test (Wald statistic = 49.62, $P < 0.001$). In week 1, the number of chickens increased with time, whereas in week 2 and 3, the number decreased (Fig. 4). In addition, the number of chicken in the vicinity of the NO was affected by the main effects of time point (Wald statistic = 17.52, $P < 0.001$) and age (Wald statistic = 21.64, $P < 0.001$), with higher numbers of chickens observed within the 25 cm radius of the NO in week 2 and 3 compared to the first week. Age effects were also found for latency of the first bird to approach (Wald statistic = 65.57, $P < 0.001$) and to touch the NO (Wald statistic = 34.11, $P < 0.001$). With increasing age, the chickens approached and touched the NO earlier (1 week of age < 2 weeks < 3 weeks).

4. Discussion

The objective of the present study was to investigate the effects of the hatching environment on general behaviour and fearfulness in broiler chickens during the entire fattening period. Therefore, direct observations (scan sampling of general behaviours) and behavioural tests were carried out in conventional hatchery-hatched chickens (C) and on-farm hatched chickens (OH) early as well as later in life. As expected, most of the observed general behaviours were affected by broiler age (Weeks et al., 2000; Bokkers and Koene, 2003). Effects of the hatching treatment were only found for a few of the general behaviours, and only observed

at some ages. In contrast, nearly all of the responses in the behavioural tests differed among treatments and ages, with C chickens and older chickens showing reduced fearfulness.

The current study represents a system comparison, which does not allow for conclusions to be drawn about single influencing factors, such as handling in the hatchery, transportation of eggs at day 18 of incubation or of day-old chickens, or the presence or absence of light during hatching and early nutrition. Conventional and on-farm hatching each include several distinct factors, which may function and interact in various ways. However, since the chickens were observed in systems and social context similar to those found on commercial farms, the present results may be comparable to practice.

4.1. General behaviours

Contrary to our hypothesis, C chickens did not show higher levels of total activity than OH chickens, neither at an early age nor later in life. We found that OH chickens had a higher body weight until day 21 of

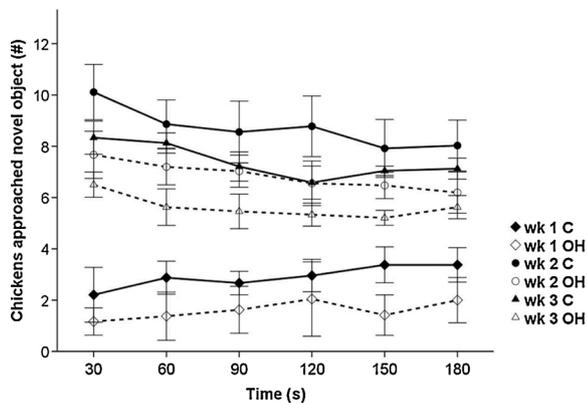


Fig. 4. Responses in the novel object (NO) test at different times during the test (30–180 s): Mean number (\pm SEM) of chickens approaching the NO at 1, 2, and 3 weeks of age (wk) for control (C) and on-farm hatched chickens (OH). Significant effects were found for treatment, age, time, and the interaction between age and time ($P < 0.05$).

age, and a lower overall mortality resulting in a slightly higher stocking density in the OH pens (De Jong et al., 2020). This could have had an additional limiting effect on general activity and then should have resulted in a larger contrast between C and OH chickens, but this was not the case. An effect of treatment was only found for disturbance behaviour, with OH chickens disturbing their pen mates more often compared to C chickens at 2, 12, and 20 days of age. However, explaining this effect is difficult. Since OH chickens showed less disturbance than C chickens before and after these time points, i.e. at 5 and 36 days of age, the effect did not seem to be consistent over the fattening period. In addition, other behavioural patterns that may be associated with disturbance, such as overall activity and aggression, did not differ between treatments, and the proportions of chickens showing disturbance behaviour (less than 2.5 %) were relatively low throughout the study period. Similar limitations seem to apply to effects we found regarding the interactions of treatment and age on eating and sitting/lying behaviour.

Thus, except for sitting/lying, disturbance and eating behaviour, the hatching environment (hatching in the hatchery vs. on-farm hatching) did not affect general behaviours of fast growing broiler chickens during

the rearing period. This may be a consequence of the only small and temporary differences in physiological development between C and OH chickens found earlier (Van de Ven et al., 2011; De Jong et al., 2019). In contrast, changes in behaviour with age seem to be more likely related to the rapid increase in body weights and the high growth potential of the broiler hybrid tested here (Weeks et al., 2000). This may have overruled smaller effects of the hatching environment on general behaviours. Previous findings show that particularly the low activity levels, especially after 3 weeks of age, are characteristic for fast growing hybrids, even though housing conditions and observation methods varied largely among studies (Bokkers and Koene, 2003; Malchow et al., 2019; van der Sluis et al., 2019). To detect smaller differences in activity and other general behaviours, more intensive behavioural observations would be required. Continuous tracking of the birds at individual (Van der Sluis et al., 2019) or group level (De Montis et al., 2013) seem to be promising approaches for future research in this field.

4.2. Fear related responses

As hypothesised, C chickens showed more active responses compared to OH chickens in a set of behavioural tests in early and later life. In the novel environment (NE) test, C chickens tended to show more flight attempts and vocalised less at d8 and thus seemed to act less fearful than OH chickens at least at d8, since inactivity and a low frequency of vocalizations during the NE test have been associated with high levels of fearfulness (Forkman et al., 2007). In contrast to our results, Hedlund et al. (2019) found more fearful responses, indicated by lower levels of activity, in a similar test situation in day-old layer chickens subjected to commercial hatchery processing compared to chickens that were not handled. However, the NE test situation consists of two distinct major features inducing fear in chickens: the experience of being captured by a potential predator (human observer) and a sudden social isolation in an unfamiliar environment (Suarez and Gallup, 1983). Therefore, responses such as vocalizations and freezing or escape attempts can be regarded as an interaction or a compromise between avoiding a predatory threat (being silent) on the one hand, and regaining social contact (showing high frequencies of vocalization) (Suarez and Gallup, 1983; Marx et al., 2001) and returning to the familiar environment on the other hand. In the present study, the frequency of vocalizations showed an interaction between age and treatment, with C chickens vocalizing more at day-old and less at 8 days of

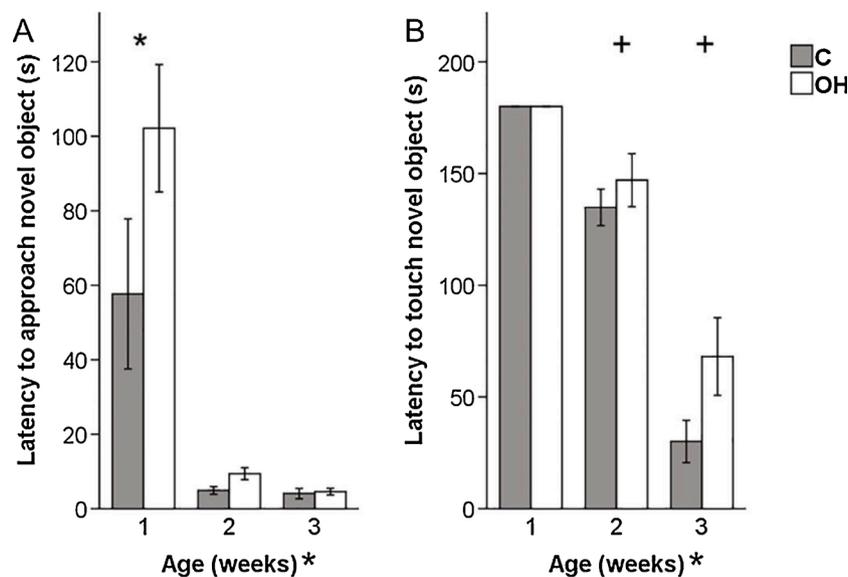


Fig. 5. Responses in the novel object (NO) test: A) mean latency of the first chicken to approach (< 25 cm) and B) to touch the novel object at 1, 2, and 3 weeks of age for control (C) and on-farm hatched chickens (OH). *Between bars denotes a treatment effect ($P < 0.05$). *After 'Age (days)' denotes an age effect ($P < 0.05$). +Between bars denotes a tendency of treatment ($P < 0.1$).

age compared with OH chickens. The results regarding C chickens are in line with previous findings suggesting that seeking social reinstatement predominates in young chickens and becomes less important with age (Suarez and Gallup, 1983). As we did not distinguish between different types of vocalizations in the present study, it is difficult to explain the higher frequency of vocalizations in OH chickens at a later age. By recording and analysing vocalization profiles of isolated chickens, Fontana et al. (2016) found that most sounds emitted at day-old could be classified as “calling sounds”, whereas the sound spectrum changed to “distress calls” at five days of age. Similarly, “distress calls” dominated in isolated 6–7 day old chickens tested by Marx et al. (2001). However, the researchers also recorded other types of vocalization in these chickens, for instance “short peeps” (Marx et al., 2001). Thus, based on the literature, the results of the present study suggest more social reinstatement in C chickens at day-old but not at eight days of age, as compared to OH chickens. Future research should not only measure the number of vocalizations during NE test situations but also analyse the type of the sounds emitted, to draw more reliable conclusions on their etiology.

In the human approach (HA) and the novel object (NO) test, which were carried out at a group level, more C chickens approached the human and the NO, and they approached them sooner as compared to OH chickens. The behaviour of the C chickens suggests less fear of humans, and of novelty, respectively (Forkman et al., 20017; Welfare Quality®, 2009). However, during complex fear related responses, it is unlikely that a specific behaviour is solely caused by one emotion, i.e. fear (Forkman, 2007). Therefore, other factors, such as exploration, imprinting, coping style, habituation and cognitive development may have influenced the chickens' behaviours. In this respect, a major challenge in interpreting the NO test is that both fearful and indifferent groups of animals may not approach the NO or show longer latencies of drawing near it (Forkman et al., 2007). The same applies to the non-forced HA test, in which, as in the present study, the voluntary behaviour of the chickens towards a stationary human is recorded (Waiblinger et al., 2006). C chickens may have had a higher motivation to search for potential feed resources, which is mirrored by the effect of treatment on the proportions of birds eating during the observations of general behaviours, and therefore showed more exploratory behaviour in the test situations compared to OH chickens. Alternatively, the responses of C chickens in the HA and the NO test may be explained by their experience with being handled by humans and being exposed to novelty during hatchery processing. The multitude of stimuli in the hatchery might elicit a complex interplay of imprinting and habituation processes in the chickens. In this sense, exposing chickens to a variety of stimuli early in life has been shown to reduce the likelihood that they will subsequently reject novel objects (Bateson, 1966). Similarly, Jones (1993) found that handled chickens, even though they had been handled roughly by suspending them by their legs, showed less avoidance behaviour towards a humans than non-handled birds. However, on the other hand, OH chickens were not reared in isolation but were exposed to various stimuli in the pen, for instance litter, light, sound emitted by the ventilation system and human contact during routine barn and animal inspections.

Coping style is defined as a set of behavioural and physiological stress responses that are relatively consistent over time and across situations. Besides the strong genetic basis of coping styles in animals, perinatal factors may also play a role in their development (Koolhaas et al., 1999). Therefore, the responses in the NE, NO and HA tests might be due to different coping styles: with C chickens having a more proactive coping style and OH chickens acting more reactively. It should be investigated further whether the chickens' physiological and neuroendocrine responses are consistent with the observed behaviours, for instance by measuring baseline levels of corticosterone and corticosterone reactivity. A low HPA axis activity and reactivity have been found to indicate a proactive coping style, whereas a normal activity and a high reactivity of the HPA axis represented a reactive coping style

(Koolhaas et al., 1999).

Moreover, Hollemans et al. (2018) suggested that a certain level of cognitive development may be a precondition for being able to express fear responses. The authors argued that delayed nutrition may lead to both impaired body and brain development. Furthermore, early nutrition may represent an early life environmental enrichment, and thus foster cognitive development including fear-related responses (Hollemans et al., 2018). Although early nutrition was only one of the factors differing between the hatching systems compared in the present study, C chickens showed a delayed body weight gain until day 21 of age and organ development on d0 (De Jong et al., 2020). Further factors affecting fear-related responses and stress susceptibility of broiler chickens later in life are light stimulation during the entire incubation period and incubation temperature (Archer and Mench, 2017; Bertin et al., 2018). However it is unknown whether light during the last 3 days of incubation and during hatching, or possible temperature fluctuations during transport of eggs at d18 of incubation (as part of the OH treatment) may have had an effect on fear-related responses and the underlying neurobiological processes. Therefore, it would be interesting to examine not only intestinal growth but also brain and neuronal development in chickens hatched in different environments. Thus, the underlying mechanisms of the differences in responses in the tests between C and OH chickens remains to be further investigated.

In both treatment groups, there were significant interactions between age and time point of observation during the HA and the NO test. At 2 and 3 weeks of age, the number of chickens approaching the human increased with testing time, whereas it stayed nearly the same in the first week. This might indicate that at a later age, when the chickens are already more accustomed with humans, a longer habituation period during the test would further reduce their fear of humans, whereas, at a young age, when general levels of fear were higher, even a longer testing time has no such effect. During the NO test, the number of chickens in the vicinity of the object increased with observation time in week 1, but decreased in week 2 and 3. This latter response might be explained by an initial decrease in fearfulness accompanied by a lack of interest in the NO once it was approached. In previous studies, different NOs were used at each testing age to avoid habituation to a certain object (Hocking et al., 2001; Van der Eijk et al., 2018). However, as the present tests were carried out at a pen level with groups of more than 1000 chickens, it is unlikely that all birds were still familiar with the NO in the following weeks. Although the underlying mechanisms are not completely clear, the present results show that general patterns of habituation or familiarisation in relation to age are present in both treatment groups – on different basal levels –, independent of the hatching environment.

5. Conclusion

The two hatching systems tested in the present study, i.e. hatching in a conventional hatchery and on-farm hatching, differed in early life environment, i.e., the availability of early nutrition, and the absence of transport of day-old chicks in the on-farm hatching environment and handling and processing in the hatchery (De Jong et al., 2017; Jacobs et al., 2017; Hollemans et al., 2018; Hedlund et al., 2019). However, the hatching system seemed to have limited effects on broiler chicken activity and the performance of general behaviours early and later in life. When the chickens were challenged in behavioural tests, hatchery-hatched chickens showed more active and less fearful responses compared to on-farm hatched chickens. To what extent this resulted from a higher intrinsic motivation to search for feed resources, from more exposure to humans and objects in the hatchery, from more effective coping strategies, or from delayed cognitive abilities to express fear related responses, remains to be studied further. Future research should therefore additionally investigate imprinting and habituation responses as well as stress indicators, such as baseline levels of corticosterone and corticosterone reactivity, and brain development in chickens hatched in these different environments.

Declaration of Competing Interest

The authors declare no conflicts of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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