

Article

Can Early Environmental Enrichment Buffer Stress from Commercial Hatchery Processing in Laying Hens?

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Abstract: Under commercial conditions, laying hen chicks are exposed to a range of stressful events immediately after hatch. Here, we studied whether environmental enrichment was able to reduce the stress sensitivity of these chicks. A total of 50 hatchery chicks (HC) and 50 control chicks (CC) were raised in enriched pens (E), while 53 HC + 53 CC were raised in standard non-enriched floor pens (NE). HC weighed less on day one, but there was no effect of hatchery treatment at later ages. HC were more pessimistic in a cognitive judgement bias test and emitted more distress calls when socially isolated, indicating that hatchery stress affected behaviour and stress sensitivity. However, enrichment did not affect the behaviour in any of these tests. We found no effects of hatchery stress in a novel environment, but indications that enrichment may have increased fearfulness of HC. The sensitivity of the hypothalamic-pituitary-adrenal (HPA) axis was reduced in HC-E compared to HC-NE, indicating that enrichment buffered the physiological stress sensitivity in HC; however, the opposite pattern was found in CC. In conclusion, our results show complex and somewhat contradictory effects on the ability of enrichment to buffer the consequences of stress in commercial hatcheries.

Keywords: hatchery stress; white leghorn; enrichment; corticosterone; behaviour; early stress



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1. Introduction

In commercial egg production, a large number of animals around the world are hatched every day under highly industrial circumstances. As previously shown, the birds are exposed to noisy incubators, and after hatch wing- or vent-sexed, vaccinated, high-speed conveyed, packed in boxes and transported to rearing farms [1]. Recently, we have shown that this causes severe stress and affects the chickens up to 25 weeks of age [1–3].

It is well known that perinatal stress has long-lasting effects on the future life of chickens, for example regarding weight and growth [4–6] as well as behaviour [7]. Stress during the period before hatch can also cause modifications to the cognitive state in animals [8]. For instance, it has been shown that high levels of corticosterone (CORT) during later part of incubation affects memory in hatchlings [9,10].

In a previous study, we showed that the stressful hatchery procedure affects cognitive judgement bias (CJB) in laying hen chicks, where chicks from a commercial hatchery tended to interpret ambiguous stimuli in a negative way, usually referred to as “pessimism” in CJB contexts [3]. However, stressed-induced negative consequences may be possible to reduce by increasing environmental complexity. It is shown that environmental enrichment can reduce effects of stress [11,12] and induce “optimistic” judgement biases [13,14] in animals. Matheson SM, Asher L and Bateson M found that wild-caught European starlings housed in an enriched environment showed a more positive CJB [15], i.e., were more optimistic, than starlings kept in a barren environment. Furthermore, removal of environmental enrichment induces a negative CJB in the starlings [16]. Environmental enrichment can also induce optimistic CJB in for example pigs [13], rats [14,17] and hamsters [18]. In chickens,

environmental enrichment has been shown to affect responses to restraint and to a novel object [19]. Hence, we hypothesise that environmental enrichment has the possibility to buffer stress from commercial hatchery procedures.

Several factors can affect stress susceptibility in chickens, such as genetic predisposition and maternal stress levels [20]. Provision of environmental enrichment can reduce fear responses, enhance exploratory behaviours and modify adreno-cortico sensitivity [21]. Jones RB and Waddington D found that chicks from enriched environments had both shorter latencies to move and engage in more locomotion during a novel arena test [21], designed to examine fear in animals [22]. During a novel arena test, individuals are not only placed in an unfamiliar environment, but they are also generally socially isolated which has the potential to induce stress. This isolation can elicit distress vocalisations (Dvoc) in young individuals that are aimed at reinstating social contact [22,23]. Moreover, the behaviours and vocalisations during social isolation model anxiety- and depression-like states, which is why young fowl are often used in anxiolytic screening research since the amount of Dvoc can be altered by administering opiate agonist or antagonist compounds [24,25].

Enrichment for chickens can be designed in several different ways, and can be defined as anything added to the home pens in order to create a more complex environment [7]. It can for example be food items, perches, dust baths and toys, but the most relevant enrichment for newly hatched chicks might be the presence of a mother hen. In nature, chicks imprint shortly after hatch on the hen and will consider her as their mother and as a conspecific. In other settings, where there is no mother hen present, they imprint on any large, moving object [26,27]. Chickens in a commercial production system are for logistical and hygienic reasons never in contact with their mother. This may have negative implications for the development of fear responses and cognitive judgement bias. For example, chickens brooded by a hen increase feeding activity and perform more ground pecking than non-brooded chicks during the rearing period [28]. There are also lower mortality levels due to cannibalism and feather pecking when the hen is present [29]. However, some of the positive effects of the mother hen can be simulated with dark brooders. For example, birds kept under dark brooders show lower levels of feather pecking [28–31], less fearful behaviour [30], and higher levels of egg production [31] compared to chickens kept in light or without brooders. Brooded chickens also seem to be less fearful to novelty [32].

In this study, we investigated if the effects of early life hatchery stress can be buffered by environmental enrichment, including imprinting on, and continuous access to, a stuffed mother hen placed on a dark brooder. We compared a hatchery stressed group of chicks with a non-stressed control group regarding cognitive judgement bias, social isolation, reactivity of the hypothalamus-pituitary-adrenal (HPA) axis, weight, and behaviour in a novel arena.

2. Materials and Methods

2.1. Ethical Note

All experimental protocols were approved by Linköping Council for Ethical Licensing of Animal Experiments, ethical permit No. 14916–2018 (Linköping, Sweden). Experiments were conducted in accordance with the approved guidelines.

2.2. Animals and Housing

The animals used in this study were White Leghorn chicks from the Lohmann LSL strain (Lohman Tierzucht, Germany). Experimental and control chicks were from the same parental flock, where all eggs were collected during the same time period. A total of 103 female chicks (hatchery chicks, HC) were hatched and handled according to standard routines in the commercial hatchery Lohmann Sverige AB to eventually be transported for 4 h by truck to Linköping University. Following hatching, they were manually sex-sorted, conveyed, vaccinated, packed in boxes, and transported for about 4 h [1]. On arrival to the university, they were placed in small arenas where they were exposed to a stuffed hen on

a turning device for 1.5 h. The reason for this was to imprint the chicks on a fake mother hen as this was used as an enrichment further on. After the imprinting procedure, chicks were weighed and leg ringed. After this, the chicks were evenly distributed into two separate rearing pens, one enriched and one non-enriched, in which they were maintained until the end of the study at five weeks of age.

The non-enriched pens were 70 × 140 cm and contained saw dust, feed, water and a heat lamp. After three weeks, the pen size was doubled, and the chicks were provided with perches. The enriched pens measured 70 × 140 cm and contained, in addition to saw dust, feed, water and a heat lamp, perches from day one including two extra high perches, a tray with peat for dust bathing and a roof heater with the stuffed hen on top and the sides covered by feathers. In addition to the regular feed, the enriched pens were provided with a muesli mix that was spread out in the pen and hidden in the peat daily. Furthermore, they were given extra feed items daily to stimulate foraging activities. This included mealworms, lettuce, corn, rose-hips, alfalfa, plums, apples, pears, bananas, cherries and blackcurrants. The enriched pens were enlarged to double the size at the same time as the control pens.

As a control group (CC), 240 fertilized eggs from the same batch as HC were collected before start of incubation. These were placed in an incubator at Linköping University at a corresponding time point as the HC, and the incubator was set at similar settings as used in the commercial hatchery. After hatching, the CC were removed from the incubator at the same time as HC. The CC were handled carefully and sex-sorted by wing inspection. The males were discarded, and the females were weighed and leg ringed. In the same manner as HC, they were exposed for 1.5 h to the stuffed hen. The CC were then evenly distributed into two separate rearing pens, one enriched and one non-enriched.

In total, there were four different treatments in this experiment: control chicks in an enriched pen (CC-E, $n = 50$), control chicks in a non-enriched pen (CC-NE, $n = 53$), hatchery handled chicks in an enriched pen (HC-E, $n = 50$) and hatchery handled chicks in a non-enriched pen (HC-NE, $n = 53$).

2.3. Weight

The chicks were weighed at hatch ($n = 206$) and at 8 ($n = 206$), 15–16 ($n = 203$), 22 ($n = 203$), 29 ($n = 203$), and 36 ($n = 202$) days of age.

2.4. Social Isolation Test

At 9 days of age, a social isolation test was performed. Social isolation is a stressful event for a young chick, and they are typically expressing this by distress vocalisations (DVoc) [23,25]. In this test, a chick was carried from its home pen and immediately placed in the test arena that was a box with solid walls measuring 58 × 35 × 41 cm with a wire mesh on top. The chick was socially isolated for 3 min and its vocalisations were recorded on a digital file during this time. The audio recordings were analysed in a custom-made Python program where sound-inputs above a threshold level are sectioned into segments separated by silence. The total amount of DVoc for a chick is calculated by the program as the total amount of segments recorded within those 3 min. Number of chicks isolated were 200 (HC-E, $n = 48$; HC-NE, $n = 53$; CC-E, $n = 46$; CC-NE, $n = 53$).

2.5. Novel Arena Test

A novel arena test was conducted at an age of two weeks. The arena measured 58 × 35 cm and contained saw dust, food, water, a novel object (an inflated blue nitrile glove), and a start box (measuring 20 × 20 × 20 cm). For the test, two chicks were randomly selected from the same home pen and placed in the start box. The test started when the sliding door to the start box was opened, and the chicks' behaviour was video recorded for 20 min. Behaviours recorded were latency to leave the start box as well as activity in the arena. As a measurement of activity, the arena was divided into four imaginary quadrants, and number of quadrant changes was used to calculate activity. In total, 203 individuals were tested in the novel arena test (HC-E, $n = 49$; HC-NE, $n = 52$; CC-E, $n = 49$; CC-NE, $n = 53$).

2.6. Cognitive Judgement Bias

A cognitive judgement bias test (CJB) was conducted on randomly selected chicks from each test pen during the 1st and 4th week of age. The arenas used for the 1st week test is shown in Figure 1. The bird to be tested was caught in its home pen and immediately placed in the start box of the arena. After 10 s, she was released from the start box and allowed to explore the arena. To gain contact with the four conspecifics that served as social attraction, the test chick had to pass a stimulus that could be perceived as positive, negative, or ambiguous at the end of an alley.

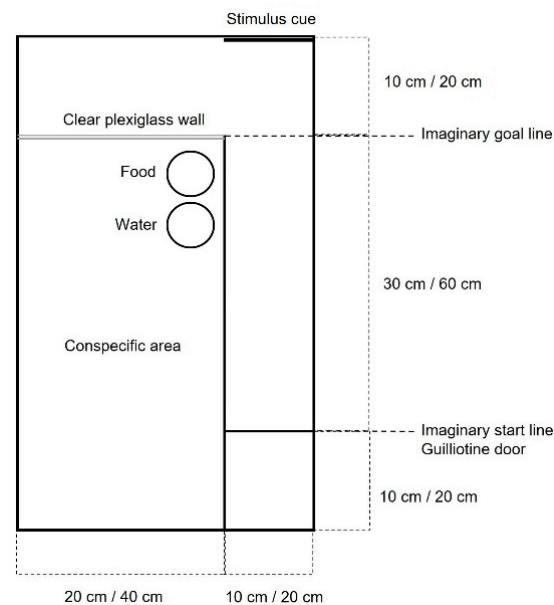


Figure 1. Arena used in cognitive judgement bias-test at one/four weeks of age. Figure reprinted with permission from [3]. Copyright 2021, copyright Louise Hedlund.

All chicks were first tested with a mirror at the end of the alley. The purpose of the mirror exposure was to serve as the most positive of the four stimuli since a mirror image is perceived as if it is a live chicken [3]. In addition to that, the mirror served as a possibility for the chicks to learn the procedure. Therefore, chicks that did not pass the goal line for the mirror within 5 min were excluded from the CJB-study. In total, at week one of age, 167 chicks passed the criteria and were tested in full (HC-NE: $n = 39$, HC-E: $n = 41$, CC-NE: $n = 41$, CC-E: $n = 46$). Out of these, 62 chicks were tested on the chick stimuli, 42 on the morph and 63 on the owl. At week four, in total 177 chicks were tested (HC-E, $n = 44$; HC-NE, $n = 44$; CC-E, $n = 45$; CC-NE, $n = 44$).

After the mirror test, all chicks were tested with one of the other three stimuli. We measured time to pass an imaginary goal line 10 cm in front of the stimulus, and for chicks that did not pass this line within 5 min, a latency of 5 min was scored. The pictures used as stimuli are shown in Figure 2. The picture of a chicken represented the most positive-like stimulus, and the owl represented the most frightening one. The most ambiguous stimulus was a morph between the chicken and the owl, created in Morpheus Photo Morpher v.3.17 Standard. All images were printed on a white background and attached to a white wall at the end of the alley. The pictures were adjusted to be the same size as the experimental chicks.



Figure 2. Pictures used for testing CJB at (A) one, and (B) four weeks of age. Figure 2A is reprinted with permission from [3]. Copyright 2021, copyright Louise Hedlund.

2.7. HPA-Axis Reactivity

At 7 days of age, a restraint test was conducted on 25 randomly selected chicks from each treatment to assess the reactivity of the hypothalamus-pituitary-adrenal (HPA) axis. Within 3 min from catching in the home pen, a blood sample was taken to establish a baseline level of corticosterone (CORT). The chicks were then restrained in a net bag for 3 min after which a second blood sample was taken to measure the increase in CORT. The blood samples were taken from the brachial vein and collected using a 200 μ L microcuvette heparin coated tube. The samples were immediately centrifuged, and the plasma was separated from the blood. They were stored in -80°C until time for analysis with a corticosterone ELISA kit from ENZO Life Sciences.

2.8. Statistics

Weight, social isolation, novel arena activity, cognitive judgement bias and restraint data were analysed with a generalised linear model with treatment (HC/CC), enrichment (E/NE) and their interaction as factors, using the normal distribution function and the link function “identity” following assessment of the suitability of the data. The test parameter Wald Chi-square ($W\chi^2$) is reported with the corresponding p -values. Tukey HSD was used to test for differences between the treatment groups. Latency to emerge from the start box in the novel arena was represented by the mean latency to emerge of the two chicks present in the same test arena. A relatively large number of birds never entered the arena, and they were given the maximum duration as a score. Since this created many truncated values, effects of treatment and enrichment on latency to emerge were analysed using Kaplan–Meier survival analysis and Cox regression analysis. All statistical analyses were done using IBM SPSS Statistics (version 28).

3. Results

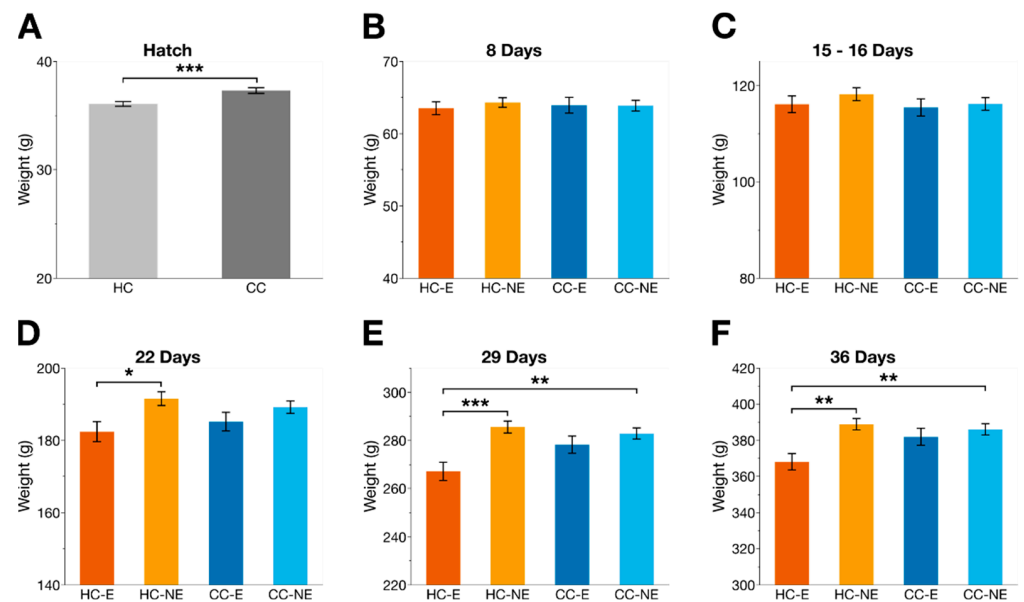
Full dataset is available in Supplementary Materials, Tables S1–S7.

3.1. Weight

Control chicks (CC) were significantly heavier at hatch than hatchery chicks (HC). After hatch, there was no significant effect of treatment on weight for the remainder of the experiment (Table 1, Figure 3). On days 22, 29 and 36 there was an enrichment effect where hatchery stressed, non-enriched chicks (HC-NE) were significantly heavier than hatchery stressed, enriched chicks (HC-E). On days 29 and 36 there was also an interaction effect between treatment and enrichment where control non-enriched chicks (CC-NE) were significantly heavier than HC-E.

Table 1. Weight of hatchery stressed chicks (HC) and control chicks (CC), enriched (E) and non-enriched (NE) chicks at different ages.

Age (Days)	Treatment (HC/CC)			Enrichment (E/NE)			Interaction Treatment × Enrichment		
	$W\chi^2$	<i>df</i>	<i>p</i> -Value	$W\chi^2$	<i>df</i>	<i>p</i> -Value	$W\chi^2$	<i>df</i>	<i>p</i> -Value
Hatch	13.73	1	<0.001	-	-	-	-	-	-
8	0.00	1	0.10	0.19	1	0.66	0.26	1	0.61
15–16	0.78	1	0.38	0.86	1	0.35	0.20	1	0.65
22	<0.01	1	0.92	8.66	1	<0.01	1.32	1	0.25
29	1.92	1	0.17	14.42	1	<0.001	5.15	1	0.02
36	2.04	1	0.15	10.49	1	0.001	4.68	1	0.03

**Figure 3.** Average weight (+/− SEM) of hatchery stressed chicks (HC) and control chicks (CC) at hatch (A) and Enriched hatchery stressed chicks (HC-E), non-enriched hatchery stressed chicks (HC-NE), enriched control chicks (CC-E) and non-enriched control chicks (CC-NE) at different ages (B–F). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

3.2. Social Isolation Test

In the social isolation test, there was a significant effect of treatment on the number of distress vocalisations (DVoc) ($W\chi^2 = 11.364$, $df = 1$, $p < 0.001$), driven by the fact that HC-E emitted more DVoc than CC-E and CC-NE. However, no significant effect was found for either enrichment ($W\chi^2 = 0.650$, $df = 1$, $p = 0.420$) or for the interaction between treatment and enrichment ($W\chi^2 = 2.410$, $df = 1$, $p = 0.121$) on DVoc (Figure 4).

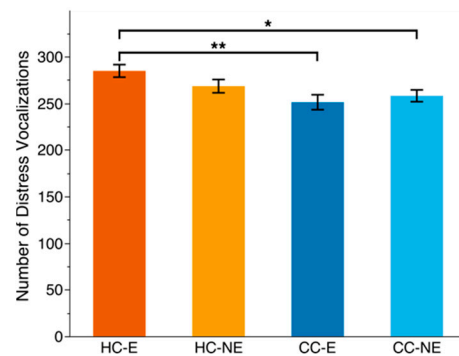


Figure 4. Mean number of distress vocalizations (\pm SEM) during social isolation in enriched hatchery stressed chicks (HC-E), non-enriched hatchery stressed chicks (HC-NE), enriched control chicks (CC-E) and non-enriched control chicks (CC-NE). * $p \leq 0.05$, ** $p \leq 0.01$.

3.3. Novel Arena Test

In the novel arena test, there was no overall effect of either treatment (HR = 0.507, 95% CI = 0.187–1.371, $p = 0.181$), enrichment (HR = 1.841, 95% CI = 0.868–3.904, $p = 0.112$) or their interaction (HR = 1.055, 95% CI = 0.307–3.626, $p = 0.932$). However, comparing each experimental group, HC-NE had a significantly shorter latency time to emerge from the start box compared to CC-E ($\chi^2 = 8.003$, $df = 1$, $p = 0.005$; Figure 5). We found no significant effect on the number of quadrant changes in the novel arena of either treatment ($W\chi^2 = 0.306$, $df = 1$, $p = 0.580$), enrichment ($W\chi^2 = 0.084$, $df = 1$, $p = 0.772$), or their interaction ($W\chi^2 = 0.825$, $df = 1$, $p = 0.364$).

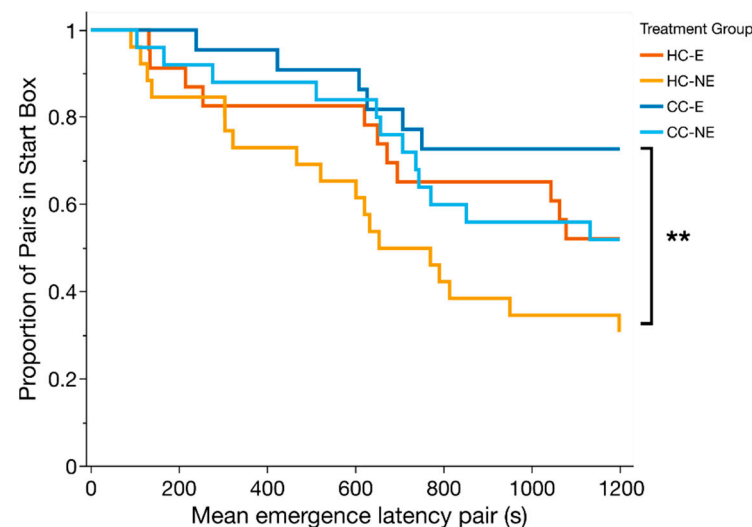


Figure 5. Survival plot showing the latencies to emerge from start box for enriched hatchery stressed chicks (HC-E), non-enriched hatchery stressed chicks (HC-NE), enriched control chicks (CC-E) and non-enriched control chicks (CC-NE) in a novel arena. ** $p \leq 0.01$.

3.4. Cognitive Judgement Bias

In the CJB test, there was a significant effect of stimulus in latency to approach at 1st ($W\chi^2 = 192.146$, $df = 3$, $p < 0.001$) and at 4th week of age ($W\chi^2 = 378.052$, $df = 3$, $p < 0.001$).

There was no significant interaction between stimuli and enrichment; therefore, this was removed from the analysis (1st week, $W\chi^2 = 1.365$, $df = 1$, $p = 0.243$; 4th week, $W\chi^2 = 0.027$, $df = 1$, $p = 0.871$). However, there was a difference between the treatments where HC were overall slower to approach the stimuli than CC. The treatment effect was significant at the 1st week of age ($W\chi^2 = 4.120$, $df = 1$, $p = 0.042$; Figure 6A) and remained as a tendency four weeks later ($W\chi^2 = 2.899$, $df = 1$, $p = 0.089$; Figure 6B). We found no

effect of enrichment, neither at 1st ($W\chi^2 = 0.290$, $df = 1$, $p = 0.590$; Figure 7A), nor at 4th ($W\chi^2 = 0.697$, $df = 1$, $p = 0.404$; Figure 7B) week of age.

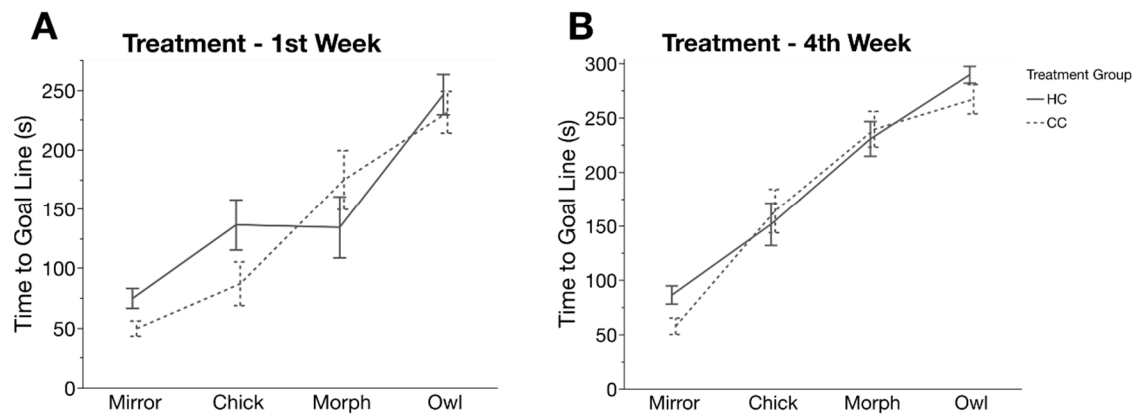


Figure 6. Mean time to reach goal line (+/– SEM) in the cognitive judgement bias test comparing hatchery (HC) and control chicks (CC) during (A) 1st week and (B) 4th week of age when exposed to a mirror, chick, morph or owl stimulus.

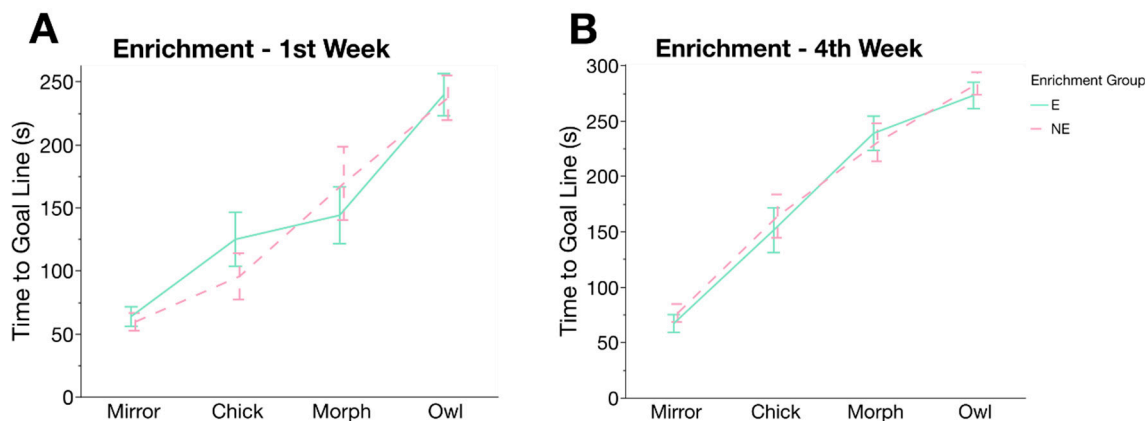


Figure 7. Mean time to reach goal line (+/– SEM) in the cognitive judgement bias test comparing enriched (E) and non-enriched chicks (NE) during (A) 1st week and (B) 4th week of age when exposed to a mirror, chick, morph, or owl stimulus.

3.5. HPA-Axis Reactivity

In the restraint test, there was no effects on baseline CORT of either treatment ($W\chi^2 = 0.296$, $df = 1$, $p = 0.587$), enrichment ($W\chi^2 = 0.237$, $df = 1$, $p = 0.626$), or the interaction between these ($W\chi^2 = 0.286$, $df = 1$, $p = 0.593$).

After restraint, there was no significant difference of either treatment ($W\chi^2 = 0.548$, $df = 1$, $p = 0.459$) or enrichment ($W\chi^2 = 0.755$, $df = 1$, $p = 0.385$). However, there was a significant effect of the interaction between these ($W\chi^2 = 3.860$, $df = 1$, $p = 0.049$). The results for the increase in CORT were in accordance with this: there was no significant difference with regards to treatment ($W\chi^2 = 0.850$, $df = 1$, $p = 0.356$) or enrichment ($W\chi^2 = 0.230$, $df = 1$, $p = 0.631$), but a significant effect of the interaction between those ($W\chi^2 = 4.473$, $df = 1$, $p = 0.034$). For the HC, enrichment was associated with the lowest CORT increase, while the opposite was the case for the CC. Results from the restraint test are shown in Figure 8.

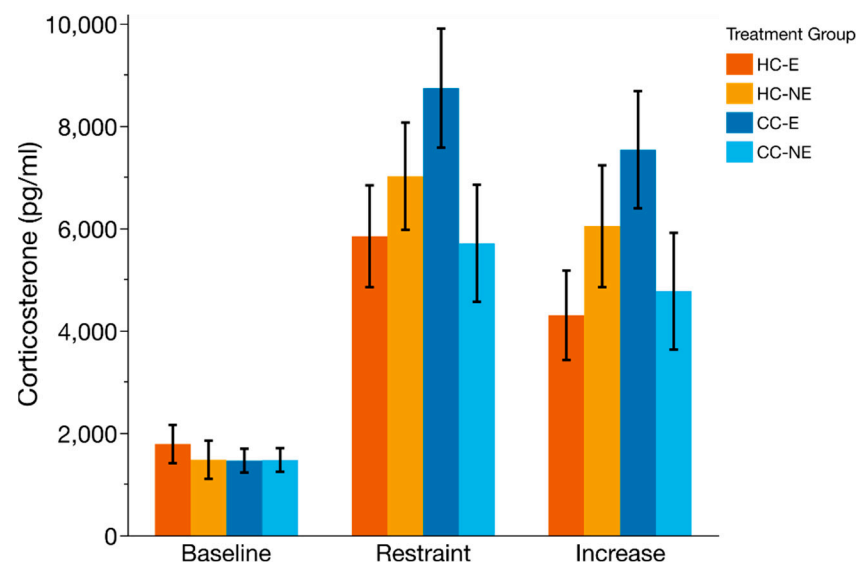


Figure 8. Mean levels of corticosterone (\pm SEM) before (baseline) and after restraint, and the increase between the two measurements, in enriched hatchery stressed chicks (HC-E), non-enriched hatchery stressed chicks (HC-NE), enriched control chicks (CC-E), and non-enriched control chicks (CC-NE).

4. Discussion

We found that early enrichment had somewhat contradictory effects on laying hen chicks hatched under stressful commercial hatchery conditions. Despite the fact that hatchery chicks (HC) weighed less at hatch, there were only minor weight differences compared to control chicks hatched under calm conditions (CC) at later ages. Chicks with enrichment (E) tended to weigh less than those in non-enriched (NE) rearing conditions. Furthermore, in a social isolation test, HC-E emitted more distress calls than CC, and in a novel environment test, HC-NE emerged faster than other chicks. Both these behaviour tests indicate that enrichment may have increased the fearfulness and stress sensitivity of the HC, contrary to expectations. HC showed a negative cognitive judgement bias, indicating a pessimistic state of mind, and enrichment did not affect this. However, HPA-axis sensitivity tended to be reduced in HC-E, whereas the opposite was found in CC, indicating that enrichment may have reduced the physiological stress sensitivity of hatchery processed chicks.

Regarding weight, we found a difference in hatch weight, but no long-lasting differences between the treatments. However, HC-groups were weighed at a later time-point than CC due to transportation delay, hence, the weight difference could at least partly be explained by dehydration. In previous studies, we have found variable effects of hatchery stress on weight development [1,33]. This may, for example, be a result of different ages of the parental flocks [34–37], something that was not controlled for in our experiments. Hatchery stressed chicks in enriched pens weighed less than those in the non-enriched pens, which was not the case for the control chicks. Our hypothesis was that chickens with enrichment would weigh more, since they had access to more feed items. One possibility is that the enrichment stimulated increased physical activity, and therefore caused a reduced weight gain, previously shown in [38]. The fact that this was seen only in the hatchery stressed chicks may indicate that they were more motivated to use the provided enrichment but should be further investigated. Previous studies have failed to show any effect of environmental enrichment on growth, but no previous study has included early stressed birds [39–43].

In the social isolation test, there was an effect of treatment where HC emitted more distress vocalisations than CC. However, this effect was mainly driven by the HC-E, indicating that enrichment actually increased the sensitivity to social isolation. Socially

isolating young chicks is well known to elicit a stress-response [44–48], and our results are therefore in line with previous studies, showing that HC display more stress-related and fearful behaviours than CC in several different contexts [1]. Possibly, the enriched chicks might have experienced more stress during the social isolation since the contrast between their home environment and the test situation was larger than for non-enriched chicks.

In the novel arena test, although there were no overall effects of either treatment or enrichment, we found that HC-NE were faster to emerge from the start-box than CC-E. This indicates that, for the HC chicks, enrichment actually increased the fear of entering a novel arena. Again, a possible explanation could be that the novel arena was more similar to the barren home environment used for the NE-chicks, which might have caused the enriched chicks to hesitate when leaving the start box. The fact that a similar effect was not observed in the CC could possibly indicate that the enrichment of the home pens had affected the HC chicks more.

In the CJB, there was an overall effect of treatment where HC appeared more pessimistic than CC, but enrichment did not have any effect in this case. This is in line with the results in a previous study in which we showed that HC were overall more pessimistic than CC [3]. Similarly, other studies have shown that exposure to temperature stress [49] or administration of CORT [50] induce negative judgment bias in chickens. The fact that we did not find any effect of enrichment on judgement bias implies that the enrichment used in this study did not affect the cognitive state of the animals. There might be other types of enrichment that are more effective for buffering of hatchery stress. It is also possible that environmental enrichment can simply not be used for this purpose, but this is something that should be further investigated.

HPA-sensitivity in the HC was reduced by enrichment, which was in line with our predictions [19,51]. However, we found the opposite pattern in the CC where enrichment tended to increase the HPA reaction to restraint. The fact that the HC responded according to our hypothesis is a strong indication that enrichment could actually buffer the consequences of early stress, since HPA-sensitivity is a central aspect of this. However, the fact that enrichment had the opposite effect on the non-stressed chicks actually confuses the interpretation and calls for more studies in order to clarify whether this is an accidental effect in the present experiment or a real effect.

A general issue with enrichment studies is the difficulty of applying the best type of enrichment. Even though enrichment is generally beneficial for animals, chicks prefer some types of enrichment over others. Skanberg L, Nielsen CBK and Keeling LJ compared different types of litter and perches and found that chicks use different materials for different purposes [52]. For example, they preferred to dust bathe in sand or peat, whereas they were most likely to engage in foraging on wood or hemp shavings. Pinchasov Y and Noy Y showed that different types of food enrichment had different effects [53], where mealworms increased short-term activity in contrast to whole wheat, while neither of these affected the reactions to tonic immobility or behavioural responses to novelty. Similarly, Baxter M, Bailie CL and O'Connell N showed that enrichment in terms of platform perches [54] and peat dust baths did not have an effect on play behaviour in broilers. In light of this, it is possible that the enrichment we have applied might not have been the most suitable in the present context.

In our experiment, all chicks—including the non-enriched ones—were exposed to the stuffed hen shortly after hatch, and possibly also imprinted on it. Hence, the non-enriched chicks were later deprived of the hen, which could have affected their behaviour and welfare negatively. There are to our knowledge no studies investigating the effect of depriving chicks from a mother they have been imprinted on. However, several studies show that the welfare of chicks benefit from having a hen present [28,55,56].

5. Conclusions

To conclude, we found some expected effects of hatchery stress such as lower hatch weight, increased stress susceptibility to social isolation and a negative judgement bias;

however, no such effects were found in the novel arena or regarding HPA-axis reactivity. Enrichment reduced the HPA-axis reactivity in the early stressed chicks but appeared to increase stressful behavioural responses in some cases. Further research should explore other means of improving the living environment in order to buffer early stress experiences in commercially hatched laying hen chicks.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/poultry1020011/s1>. Table S1. CJB 1st week; Table S2. CJB 4th week; Table S3. CORT during restraint; Table S4. Weight; Table S5. Novel arena activity; Table S6. Novel arena emergence; Table S7. Social isolation.

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