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Early life environment and adult enrichment: Effects on fearfulness in laying hens

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ABSTRACT

The environmental complexity, both during early and adult life, contributes to shaping individuals' fearfulness. The present study aimed at testing whether hens reared in an aviary were less fearful than hens reared in cages, and whether provision of additional enrichment during the laying phase could reduce fearfulness. We used White Leghorn laying hens (N = 384) reared in cages (N = 192) or in an aviary (N = 192) and then housed in furnished cages from 18 weeks of age, with or without the provision of additional enrichment. We tested naïve hens at 31 and 60 weeks of age in a novel object test and at 33 and 61 weeks of age in an open field test. Cage-reared hens had a latency to approach the novel object comparable to the one of aviary-reared hens when tested at 31 weeks of age ($F_{1, 17} = 2.71$; $p = 0.12$). At 60 weeks of age, birds housed in additionally enriched furnished cages were significantly faster to approach a novel object than birds housed in standard furnished cages for both rearing conditions ($F_{1, 61} = 19.02$; $p < 0.01$). Hens reared in cages walked distances comparable to aviary-reared hens in the open field arena at 33 and 61 weeks of age ($t = -0.33$; $p = 0.75$ and X^2 (1, N = 123) = 0.02; $p = 0.89$, respectively), and the provision of additional enrichment during the laying phase did not increase that distance (X^2 (1, N = 123) = 2.01; $p = 0.16$). We also did not observe any differences in the latency to start moving in the arena ($p > 0.05$). These results suggest that the environmental complexity during rearing had no medium- and long-term effects on fearfulness measured in the open field and novel object test. However, additional environmental enrichment during the laying phase had a stronger influence, reducing fearfulness towards novelty. This study suggests that environmental enrichment during adulthood can have positive effects on laying hens' fearfulness.

1. Introduction

Fearfulness can be defined as the individual 's predisposition to be easily frightened (Boissy, 1995; Jones et al., 1996). This trait is important to protect the animal from danger but can decrease welfare if responses to fear-inducing stimuli are disproportionate (Mills and Faure, 1990; Boissy, 1995; Jones et al., 1996). In farm animals in general, increased fearfulness is known to lead to difficulty in handling the animals and loss of productivity (Boissy and Erhard, 2014). In laying hens, increased fearfulness can lead to feather pecking (de Haas et al., 2014), smothering (Gilani et al., 2012) and to a higher risk of keel bone fracture (Harlander-Matauschek et al., 2015). In addition, fear is also associated with negative emotional states, which can in turn affect animal welfare negatively (Boissy, 1995).

The environment during early life contributes to preparing the individual to its future life (Bateson et al., 2014). During that time, the brain is very plastic and neuronal circuits are shaped to adapt to the current environment. This can have long lasting effects on neurophysiology and behaviour (Di Segni et al., 2018), notably with regards to fearfulness and response to novelty (Caldji et al., 2000; Pryce et al., 2005). For example, a more complex environment during early life has been shown to decrease individuals' fearfulness in rodents (Peña et al., 2009), pigs (Beattie et al., 2000) and broiler chickens (Tahamtani et al., 2018).

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In laying hens, the production system makes a clear division between early and adult life. Hens are normally reared in a rearing farm before being transferred to the laying facilities before the onset of lay at around 16–18 weeks of age. This life stage division makes laying hens well suited for research on how early and adult experience shape the behaviour and physiology of the individual. In the EU, battery cages are prohibited, and the industry is moving towards cage-free systems. Worldwide, however, cage housing is still prevalent, especially in the pullet phase (Schuck-Paim and Alonso, 2021). These two rearing environments are interesting as they present two distinct levels of environmental complexity. Individuals reared in cages grow in a relatively poor environment, with few opportunities to express natural behaviours and develop cognitive abilities. The aviary rearing system offers more opportunities to the individual with, among other things, the option to navigate in three-dimensional space and the option to perform more locomotor behaviours such as stretching. The difference in rearing systems can lead to individuals with different traits and behavioural phenotypes (Tahamtani et al., 2014, 2015; Brantsæter et al., 2016a).

Most studies have looked at short-term effects of rearing on adult behaviour, but the effects of early life environment, probably due to effect on the developing nervous system, could potentially have long lasting impact on the behaviour. Although early-life experiences are normally thought to have crucial impact on behavioural development, later-life environment may modulate these effects (Nicol et al., 2001). For example, the provision of environmental enrichment during the peripubertal period or adulthood has been shown to reduce anxiety in rats (Francis et al., 2002; Koe et al., 2016). While the understanding of later-life environment modulating the effects of early-life experience is of basic interest, it also has implication for the laying hen industry as exaggerated fearfulness responses can cause damage to the birds both in cage and free-range systems (Jones, 1996). We therefore aimed to explore both medium- and long-term effects of the rearing environment, and the effect of environmental enrichment provision during the laying phase, on fearfulness. We compared behaviours of individuals reared in cages or in an aviary and then transferred to furnished cages, with or without the provision of additional enrichment. Data was collected within a few months after birds were transferred to furnished cages at the production farm and again after several months of housing in additionally enriched or standard Victorsson T10 furnished cages. Because aviaries represent a more complex and stimulating environment, we predicted that hens reared in an aviary would be less fearful than hens reared in cages. We also predicted that enriched housing during the laying phase would partially compensate for the effects of the rearing environment so that birds reared in cages but provided with enrichment as adults would be less fearful than birds reared in cages and housed without enrichment.

2. Material and methods

2.1. Animals, rearing and housing

2.1.1. Rearing

This study was conducted using 384 Lohmann White Leghorn hens. The birds were either reared in an aviary (N = 192) or in cages (N = 192) until transport to the experimental farm at 18 weeks of age. All birds were reared in one single room measuring 15 m x 72 m at a commercial hatchery (Steinsland & Co.). The room contained 38,000 birds housed in raised NATURA Primus 16 system (Big Dutchman, www.bigdutchman.com, see Fig. 1). Cages measuring 12 m × 0.8 m × 0.6 m (length × height × width) were stacked in three tiers. Each tier contained a feed line and nipple drinkers. After hatching, birds were placed in the first and second tier of the system. The front of all cages was closed, and the floor of the cages was lined with paper until 4 weeks of age. For birds in the cage-reared condition, the front of the cage remained closed during the whole rearing period to simulate cage rearing. The cage was located in the second tier of one of the aviary rows and contained 250 birds. In the aviary-reared condition, the front of the cages in the first and second tier was opened from five weeks of age. Birds could move freely throughout the whole room by navigating through, over, or under the aviary tiers. Wood shavings were used as litter material on the floor of the room, and perches were available in the aviary rows over the water line and the feed line. Once the front of the cage was open, birds had access to perches on the front of the cage. Additional perches were also extended from the cage front at seven weeks of age (see Fig. 1).

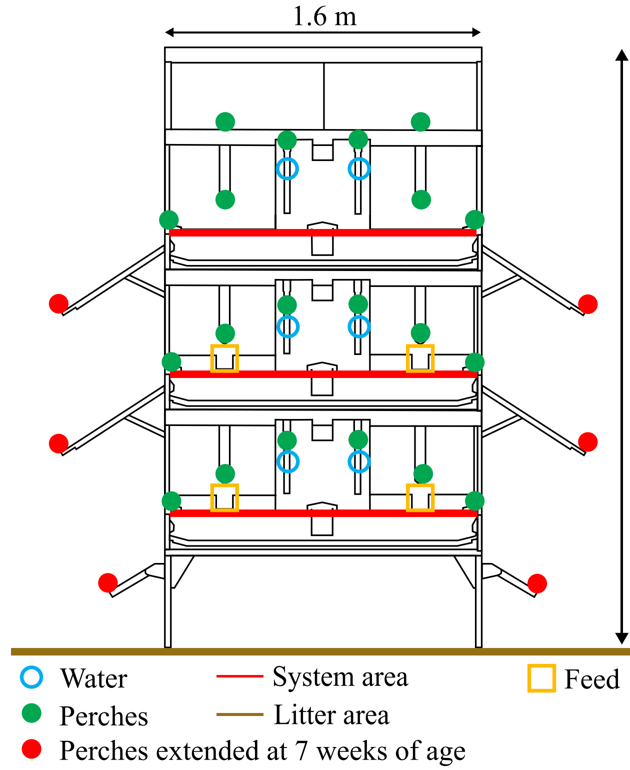

Fig. 1. Schema of Natura Primus 1600 viewed from the end of the row showing feed lines, water lines, and perches (based on the Big Dutchman leaflet).

From 5 weeks of age, the density was 26 birds/m^2 for the cage-reared birds and 29 birds/m^2 for the aviary-reared birds. All birds were exposed to the same lighting and feeding schedule. Temperature started at 34 °C and was gradually decreased to 19 °C at 16 weeks of age. Birds were exposed to 24 h of light for the first day, followed by a continuous 4:2 light/dark cycle during the first week as recommended by the Lohman LSL management guide. The light schedule was then switched to 16:8 light/dark at two weeks of age and gradually decreased to 9:15 light/dark by five weeks of age. Gradual transitions from dark to light and from light to dark were used. Each transition took 20 min. All birds received vaccination against coccidosis and Marek's disease.

At 18 weeks of age, 192 birds were randomly selected from the aviary (aviary-reared birds) and 192 birds were randomly selected from the tier which was kept closed (cage-reared birds).

2.1.2. Adult housing at the experimental farm

2.1.2.1. Description of the experimental facility. The henhouse contained 2808 cages organised in 12 rows. Each row contained 6 tiers, with a walkway between the 3rd and 4th tier, thus forming two floors in the building. The cages used for housing experimental birds were all situated on the third tier of the second floor, i.e. in the top tier. The four birds sharing a cage came from the same rearing treatment. The distribution of treatments in cages was balanced so that cages with birds reared in the aviary were always next to cages with birds reared in cages.

2.1.2.2. Type of housing during lay. After the arrival of the birds at the experimental farm at 18 weeks of age, they were housed in social groups of four in two Victorsson T10 cages that were adjoined by an opening measuring 15 cm × 18 cm. Each pair of cages containing four birds is hereafter referred to as a cage. Each cage measured 240 cm × 83 cm × 63 cm (width × height × depth). Standard control cages (n = 64) were furnished with two nest boxes, four perches and two metal dustbathing trays on the roof of each nest box (Fig. 2). Additionally enriched cages (n = 32) were the same as standard control cages with the addition of an extra dustbathing tray for stimulating foraging and dustbathing, a hemp pompon to peck at, and polyethylene tarp curtains and sheets to increase structural complexity. The latter were hung under one of the perches of the cage. In addition to this, a low-density polyethylene (LDPE) sheet was hung on the upper edge of each opening between the two cage-halves. Birds could therefore not see past these barriers and either had to move under or around them or push them out of the way to move past them. The extra dustbathing tray (55 cm × 60 cm width × depth with a 2 cm high frame to keep dustbathing material from falling off the tray) was placed on the perches in one half of the cage and refilled weekly with a mixture of feed crumbles and dustbathing pellets made of pelleted wheat husks. The pompon was attached to the cage front above the dustbathing platform so that it hung at the upper half of the cage wall. To slightly increase environmental variability, the position of the dustbathing platform and the pompon was switched to the opposite side of the cage every two months starting when the hens were 42 weeks old.

All birds were exposed to the same lighting and feeding schedule during their time at the farm. From the age of 18 weeks, they were kept under a 13:11 light/dark cycle and a temperature of 21.1 ± 1.6 °C without exposure to additional daylight from the outside. Gradual transitions from dark to light and from light to dark were used. Each transition took 15 min. Food and water were provided ad libitum via a food chain running in front of the cages and a water line with nipple drinkers along the back of the cages. For identification purposes, each bird was individually marked by means of a black or white plastic zip-tie around its left or right leg.

2.1.2.3. Experimental design. In total, 128 birds from each rearing environment were housed in standard furnished cages and 64 birds from each rearing environment were housed in additionally enriched furnished cages (see Fig. 3). The birds reared in cages or in an aviary and housed in standard furnished cages will be referred to as cage standard (cS) and aviary standard (aS). The birds housed in additionally enriched furnished cages will be referred as cage enriched (cE) and aviary enriched (aE). Half of the cS and aS birds (n = 16 cages/treatment groups, Fig. 3) were tested to study the medium-term effects of the rearing environment between 31 and 34 weeks of age. As part of another experiment, one bird per cage was removed at 24 weeks of age. The birds used to study the medium-term effects of the rearing environment were thus housed in groups of three from that age on. The other half of the cS and aS birds (n = 16 cages/treatment groups, Fig. 3) were tested along with the cE and aE birds (n = 16 cages/treatment groups, Fig. 3) to study the interaction effect of the rearing environment with the laying environment between 60 and 61 weeks of age.

2.2. Novel object test

A first novel object test was performed on hens housed in standard furnished cages at 31 weeks of age. The test was performed in the home cage of the birds. Birds in sixteen cages from each rearing condition were tested. At the beginning of the test, all hens from the same cage were gently moved to one half of the cage. A small eggcup glued onto a plywood square (19 cm × 19 cm) was baited with 3 mealworms (*Tenebrio molitor*; (Invertapro, Voss, Norway); known as a palatable food reward for hens; Moe et al., 2009) and placed in the empty part of the cage. The cup was placed in the middle of the cage-half used for testing and was visible from the other cage-half containing the birds. The food reward was visible once the hen entered the cage half containing the cup. The experimenter moved as far away from the cup as possible (~1.5 m) and started scoring. The latency to the first peck at the cup and total number of pecks at the cup were recorded. Considering the low occurrence of pecking behaviour observed, the latency to enter the cage-half with the cup (both legs had crossed the door between the two parts of the cage) was added to the list of variables recorded for nine cages of cage-reared birds and ten cages of aviary-reared birds. These variables were recorded at cage level, and the identity of the hen entering the cage-half with the novel object and being the first to peck

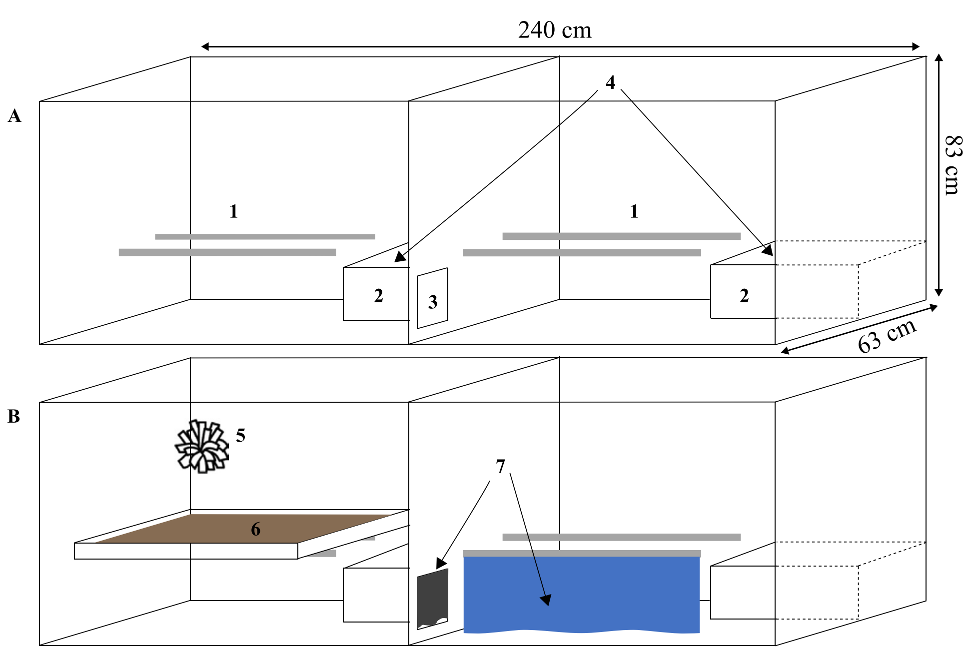

Fig. 2. Schemas of a standard Victorsson T10 furnished cage (A) and an additionally enriched Victorsson T10 furnished cage (B), three-quarter front view, showing (**1**) the perches, (**2**) the nest boxes, (**3**) the opening between the two parts of the cage, (**4**) the dustbathing trays, (**5**) the hemp pompom, (**6**) the additional dustbathing tray and (**7**) the curtains. The features **1**–**4** were also accessible in the additionally enriched cages.

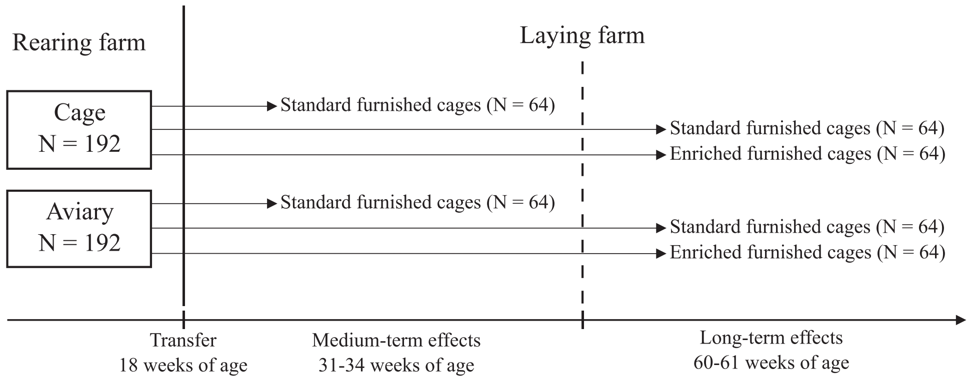

Fig. 3. Distribution of individuals in the different types of housing. A subset of bird was tested at 30–33 weeks of age and the other part at 60–61 weeks of age.

was not used in further analysis. The test was stopped after 5 min and the cup removed from the cage.

The novel object test was also performed on a different group of hens housed in standard furnished cages and additionally enriched furnished cages at 60 weeks of age. Birds from sixteen cages from each treatment group (aE, aS, cE, cS) were tested. The curtain between the two cage-halves was removed from the additionally enriched cages to ensure that the novel object was visible. The procedure followed was otherwise the same as the one described in the previous paragraph.

2.3. *Open field test*

The test was performed at 33–34 weeks of age on cage- or aviary-reared hens and at 61 weeks of age on different hens from the four treatment combinations (aE, aS, cE, cS). The two hens tested at each age were picked from the cages previously tested in the novel object test. The tests were conducted in two temporary arenas built in the hen house. In each arena, three of the walls were made of wood frames covered by a dark tarp, the fourth wall being the wall of the building in grey cement. The floor was made of particle board. Each arena measured 350 cm × 177 cm and walls were of 190 cm height. Lighting was provided by two lamps, one per arena, mounted on one of the walls of the arena. One camera (Axis m1124-e network camera, Noldus, The Netherlands) was mounted on the cement wall over each arena, at approximately 2.5 m of height, to allow the recording of the trials. All trials were recorded using the MediaRecorder system (Noldus Information Technology, Wageningen, The Netherlands).

At 33–34 weeks of age, 30 birds from each treatment group were tested. Two birds from the same cage were transported to their respective arenas and tested alone, one bird per arena, at the same time. The hen was placed in one of the corners of the arena and the test lasted for 10 min. At the end of the test, each arena was swept clean, and the hens were returned to their home pen. The corner of the arena used as the start point was the same for all tests.

For the second round of testing, naïve hens were 61 weeks of age (cS: N = 30; cE: N = 31; aS: N = 30; aE: N = 32). The hens were placed in a start box which was lifted by the experimenter from the outside of the arena to synchronise the start of the trials in the two arenas. The start box consisted of a grey plastic box measuring 40 cm × 30 cm × 20 cm (length × width × height) turned upside down. The procedure was otherwise the same as the one described for the first round of testing.

Videos were analysed using EthoVision X9 (Noldus Information Technology, Wageningen, The Netherlands). The latency to move and the total distance moved were recorded. The latency to move was defined as the central point of the hen's body crossing the line of the start area. The start area was a zone of approximately 40 cm × 40 cm in the corner of the arena where the hen was placed at the beginning of the test. The total distance moved was estimated by tracking movement of the central point of the hen at a rate of five samples per second. The sample point was set at the previous location until the distance moved was more than 5 cm to prevent an overestimation of the total distance moved. The track was also smoothened based on five samples before and after the sample point.

2.4. *Data analysis*

All statistical analysis were performed with R, version 4.0.3 (R Core Team, 2021). We used linear (mixed effects) models (L(M)Ms) fitted by restricted maximum likelihood estimates. Details on the structure of each model can be found below, under the subheadings of the different tests. P values were calculated by Wald chi-square and Wald F-test. All models were checked for assumptions (homogeneity of variances and normal distribution of residuals) and raw data were transformed to fit the assumptions when necessary. Interactions between predictors were first included in the models. When not significant, the interaction was removed, and the model was run again. In the case of one of the main effects being significant, within groups comparisons were performed by running the model on a subset of the dataset.

2.4.1. *Novel object test*

Due to a very low occurrence of pecking on the cup (13 cases over the 83 cages tested during the two tests), only the latency for the first bird to enter the cage-half containing the cup was analysed.

For each round of testing (age = 31 or 60 weeks), very few cages had the maximal cut-off latency of 300 s (two and one cage, respectively), so we used a LM in place of a survival analysis. The latency for the first bird to enter the cage-half with the novel object was used as the response variable (data were root transformed to meet the assumptions of the LM) and the type of rearing environment as a predictor. At 60 weeks, the provision of enrichment during laying was added in the model as a predictor on its own and in the interaction with the type of rearing. However, the interaction between the rearing and laying environments was not significant ($p > 0.05$) and was thus removed from the model. The whole cage was used as the statistical unit.

2.4.2. *Open field test*

The total distance moved and the latency to move were used as response variables. The data were root transformed to meet the assumptions of the LMM. The rearing environment was used as a predictor, and the cage was used as a random factor to account for the lack of independence between hens from the same cage. For the second round of testing (age = 61 weeks), the laying environment (additionally enriched or not) was added in the model as a predictor and in the interaction with the type of rearing. However, as for the novel object test, the interaction between the rearing and laying environments was not significant ($p > 0.05$) and was thus removed from the model.

The total distance moved from the first round of testing (hens aged of 33 weeks) did not meet the homogeneity of variances criterion. The values for each cage were thus averaged, and a Welch t-test for non-homogeneous variances was used in place of the LMM.

2.5. Ethical statement

The animals used in this study were enroled in a larger project. An application for permission to perform the animal studies was submitted to and approved by the Norwegian Food Safety Authority (FOTS ID 22443). The experiments were performed in a farm approved as an experimental facility, and the experimental hens were housed in compliance with the Norwegian legislation regarding the use of animals in research (Forskrift om bruk av dyr i forsøk).

3. Results

3.1. Novel object test

At 31 weeks of age, there was no significant difference in the latency to enter the cage-half with the novel object between hens reared in cages or in an aviary ($F_{1, 17} = 2.71$; $p = 0.12$; cage-reared: 131 s ± 35 s; aviary-reared: 72 s ± 25 s, Fig. 4A).

At 60 weeks of age, hens housed in standard furnished cages were significantly slower to enter the cage-half with the novel object than hens housed in additionally enriched furnished cages ($F_{1, 61} = 19.02$; $p < 0.001$; standard: 81 s ± 14 s; enriched: 27 s ± 4 s, Fig. 4C). This difference was significant both within the cage-reared ($F_{1, 30} = 9.7705$; $p < 0.01$; cS: 90 s ± 23 s; cE: 26 s ± 6 s, Fig. 4C) and the aviary-reared ($F_{1, 30} = 9.6384$; $p < 0.01$; aS: 72 s ± 16 s; aE: 28 s ± 5 s, Fig. 4C) groups. The rearing environment had no significant effect on the latency to enter the cage ($F_{1, 61} = 0.09$; $p = 0.76$; cage-reared: 58 s ± 13 s; aviary-reared: 45 s ± 9 s, Fig. 4B).

3.2. Open field test

There was no significant difference in the distance moved between the different treatment groups at 33 weeks of age ($t = -0.33$; $p = 0.75$; cage-reared: 8.95 m ± 1.67 m; aviary-reared: 8.32 m ± 1 m, Fig. 5A). At 61 weeks of age neither the rearing environment (X^2 (1, N = 123) = 0.02; $p = 0.89$; cage-reared: 8.57 m ± 1.03 m; aviary-reared: 8.59 m ± 1.04 m, Fig. 5B) nor the adult environment (X^2 (1, N = 123) = 2.01; $p = 0.16$; standard: 7.13 m ± 0.84 m; enriched: 9.96 m ± 1.16 m, Fig. 5C) had an effect on the total distance walked in the arena.

Cage-reared hens and aviary-reared hens also did not significantly differ in their latency to start moving at 33 weeks of age (X^2 (1, N = 60) = 1.21; $p = 0.27$; cage-reared: 269 s ± 37 s; aviary-reared: 209 s ± 31 s, Fig. 6A) or at 61 weeks of age (X^2 (1, N = 123) = 2.27; $p = 0.13$; cage-reared: 304 s ± 26 s; aviary-reared: 251 s ± 26 s, Fig. 6B). The provision of enrichment had no significant effect at 61 weeks of age either (X^2 (1, N = 123) = 1.20; $p = 0.27$; standard: 297 s s ± 26 s; enriched: 259 s ± 26 s, Fig. 6C).

4. Discussion

The aim of the experiment discussed here was to get a better understanding of the effects of environmental complexity during rearing and the laying phase on laying hens' fearfulness. Because of the known positive effects of environmental complexity on the developing and adult individual (Beattie et al., 2000; Francis et al., 2002), we predicted that hens reared in an aviary would be less fearful than hens reared in cages. We also predicted that exposure to additional enrichment during the laying phase would partially compensate for the effects of the rearing environment. The results show that the effects were not consistent across the tests and that environmental complexity affects fear responses differently depending on the test method chosen.

Effects of early life environmental complexity have mostly been studied with focus on the short-term effects. In a previous study using a similar housing design, hens reared in an aviary spent more time in the zones close to the novel object than cage-reared hens five weeks after transfer to furnished cages (Brantsæter et al., 2016b). However, the duration of this rearing effect was not investigated. Contrary to Brantsæter et al. (2016b), we found no significant differences between cage- and aviary-reared hens in the latency to approach the novel object at 31 and 60 weeks of age. At 31 weeks of age, there was still a trend going in the same direction as the findings from Brantsæter et al. (2016b), with aviary-reared hens showing a shorter latency to approach the novel object. This trend was not present at 60 weeks of age, i.e., 42 weeks after transfer to the laying farm. This suggest that the effects of the complexity of the rearing environment fade over time, starting already after 13 weeks of transfer to the laying farm. A study by Hocking et al. (2001) showed that the birds avoided the novel object less as they aged. The lack of difference in the latency to approach the novel object between the cage-reared and aviary-reared hens could thus also be due to the age of the individuals, and not only to the effects of the rearing environment fading over time.

In a recent study, the current provision of environmental enrichment to hens housed in aviary systems had no effects on behaviour in the novel object test (Tahamtani et al., 2022). In contrast, our study showed that the provision of environmental enrichment during the laying phase significantly decreased the fearfulness of the hens when exposed to a novel object at 60 weeks of age. Hens housed in additionally enriched furnished cages were significantly faster than hens housed in standard furnished cages to approach the novel stimulus independently of the rearing condition. The inconsistency in results may be due to the fact that the hens used in our study were housed in furnished cages, which

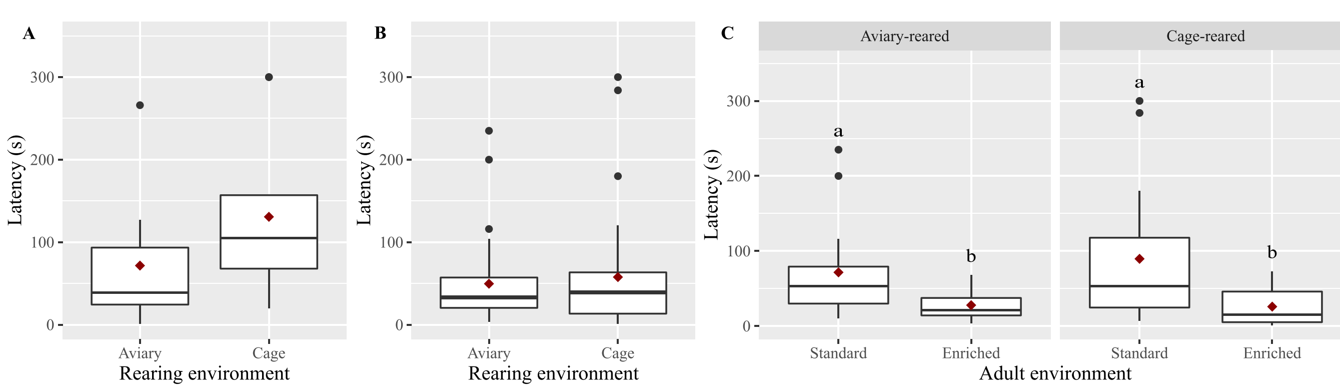

Fig. 4. Latency to enter the cage-half with the novel object at 31 weeks (A) and 60 weeks (B) of age for both rearing environments. The graph C shows the latency to enter the cage with the novel object at 61 weeks of age for each combination of rearing and adult environment. [a-b] Bars with no common letters differ significantly ($p < 0.05$).

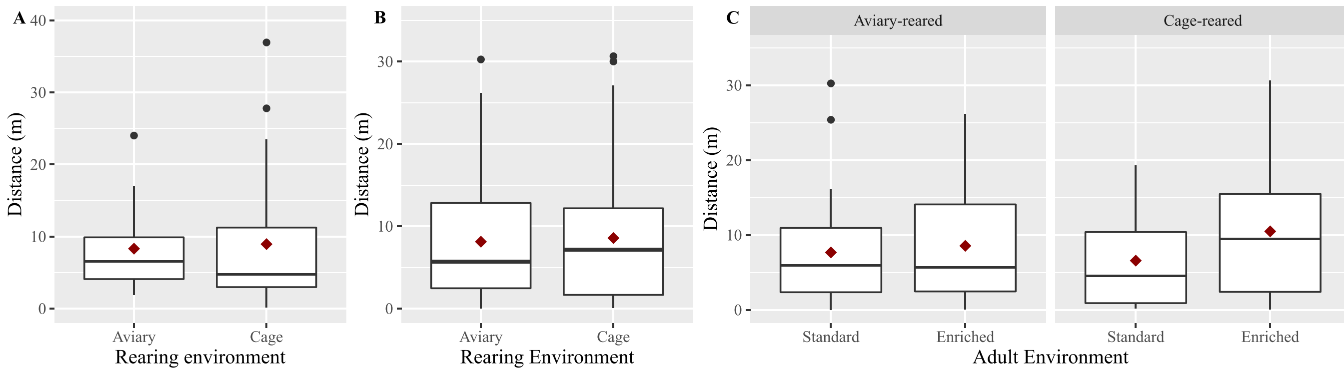

Fig. 5. Total distance moved during the open field in metres at 33 weeks (A) and 61 weeks (B) of age for the two rearing environments. The graph C shows the total distance moved during the test at 61 weeks of age for each combination of rearing and adult environment.

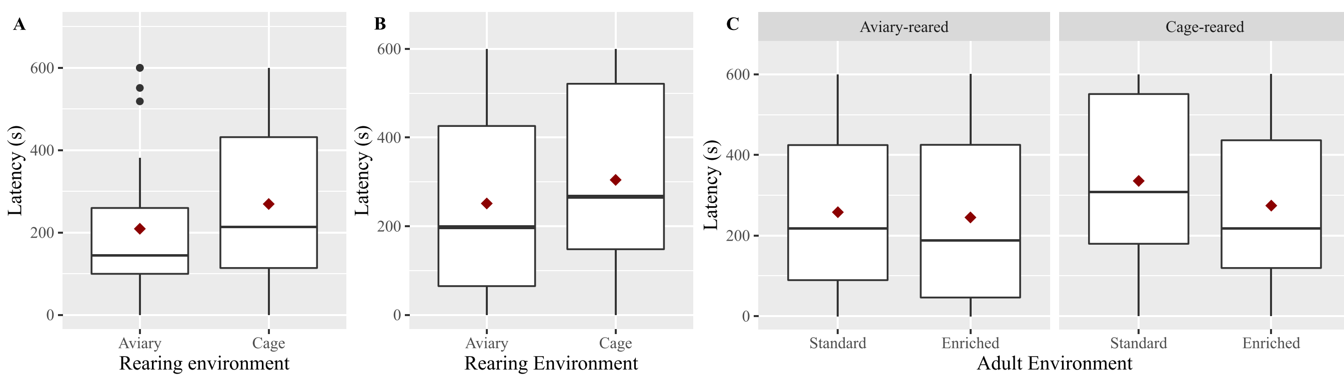

Fig. 6. Latency to start moving during the open field test in seconds at 33 weeks (A) and 61 weeks (B) of age for the two rearing environments. The graph C shows the total latency to start moving during the test at 61 weeks of age for each combination of rearing and adult environment.

represent a less complex environment than the aviary system studied by Tahamtani et al. (2022). The provision of environmental enrichment could thus be more beneficial for birds housed in cages and have a stronger impact as they face less stimulation than birds housed in more complex systems, such as aviaries.

The effects of environmental complexity and enrichment on the results of the open field test are not consistent with the results from the novel object test. Contrary to our predictions, we did not find any effects of the environmental complexity during rearing on the total distance moved in the open field test at 31 weeks of age. Hens reared in cages did not walk less in the open field than hens reared in an aviary, but showed the same level of exploratory behaviour. Increasing the environmental complexity during laying by providing additional environmental enrichment in the cage had no clear effects on the latency to move, nor on the total distance walked in the open field arena. The aviary-reared hens in our experiment came from an environment rich in stimulation but were transferred to furnished cages. Though the provision of enrichment increases the complexity of the cage and allows for the expression of more behaviours, it is still quite limited and represents a less complex environment than an aviary. That change from a more complex to a less complex environment could lead to frustration, as the birds are more restricted in their behaviours, and depression. For example, rats losing access to enrichment have been shown to express more depression-like behaviours than the control group (Morano et al., 2019; Smith et al., 2017). The results of our study could thus be affected by the loss of environmental complexity, which could lead to the aviary-reared hens not showing the expected higher degree of exploratory behaviour.

In laying hens, higher latency to move and reduced locomotion in an open field test are commonly used as indicators of higher fearfulness (Forkman et al., 2007). However, not all studies document the expected differences in fearfulness in this test. In Nordquist et al. (2011), chicks from two different lines were tested in a battery of tests to assess, among other things, fearfulness and anxiety. No differences were found between control and low mortality lines in the open field test, despite a difference in behaviour in the voluntary approach test. This is consistent with our results, failing to show any differences in the levels of fearfulness measured in the open field test despite clear differences between birds from the different treatment groups in the novel object test. Several factors might explain these differences.

First, birds must be transported from their home pen to the testing arena to be tested in the open field. The handling and transport, though gentle, can increase the stress levels of the birds and bias the measures taken during the open field test. Indeed, Fraisse and Cockrem (2006) and Littin and Cockrem (2001) studied plasma corticosterone concentration of hens in response to repeated handling. They both showed that repeated handling during 15 min was enough to elicit an increase in plasma corticosterone. This could lead to higher fearfulness. In Marin et al. (2001), chicks were exposed to acute stress before being tested in a tonic immobility test. Chicks subjected to acute stress before testing had a longer duration of tonic immobility than the control group, suggesting that acute stress induces higher underlying fear levels.

Another factor which could explain the difference in the levels of fearfulness measured between the open field and the novel object tests is the social environment. Indeed, those two tests as used in the current study measure fearfulness at the individual and group levels, respectively. Taking the measure at the cage level during the novel object test only reflects the latency to approach the novel object for the bravest bird of the cage. There is thus a loss of information on individual variability, and taking measures of more than one individual per cage might show more differences between the treatment groups. In addition to that, chickens are social animals, and social isolation can increase stress levels. For example, socially isolated quail showed increased plasma levels of corticosterone even when isolated in a familiar environment (Hazard et al., 2008). Social isolation could increase the fear levels prior to testing to a level at which differences between the treatment groups are not noticeable anymore. The fear indicators measured in the open field are therefore the response to a novel environment, but also to social isolation. In contrast, the novel object test as used in the current study takes a group level measure of fearfulness since birds are tested directly in their home pen with familiar conspecifics. The output measure is thus the fear response to the novel object and is not affected by a change in the social environment. In commercial settings, individuals are rarely isolated and so measures at the group level might therefore be more relevant.

5. Conclusion

In conclusion, we found that rearing hens in different levels of environmental complexity had no medium- or long-term effects on the fearfulness measured in an open field and a novel object test. However, the provision of additional enrichment during the laying phase reduced fearfulness towards a novel object. These results suggest that environmental enrichment during adulthood can have positive effects on laying hens' fearfulness.

Declaration of Competing Interest

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

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