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Effects of the rearing environment complexity on laying hens' spatial cognition: A holeboard test approach

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ABSTRACT

The rearing environment of layer chicks can differ greatly in degree of complexity. With the industry moving towards cage-free housing systems, greater demands are placed on the birds' cognitive abilities in order for them to find resources such as food, water and nest-boxes. Because early environmental complexity can influence cognition, we aimed at increasing our knowledge of how two different rearing environments affect the cognitive abilities of the hens. We habituated 64 hens to a spatial holeboard test, half of which were reared in cages and the other half in an aviary. Out of these 64 hens, 14 cage- and 14 aviary-reared White Leghorn hens were tested twice a day every workday in a holeboard test from 32 to 40 weeks of age. The test consisted of 4 consecutive phases, namely the uncued, cued, over-training and reversal phases, during which the hens had to find baits in a subset of cups in an arena. All cups were identical, so hens had to rely on spatial cues to find the baits which were always hidden in the same cups. During the cued phase, cues were added to the baited cups to give additional information to the hens. During the reversal phase, baits were hidden in a new subset of cups to study cognitive flexibility. The results show the birds were able to successfully complete the task. Aviary-reared hens had a higher reference memory score than cage-reared hens in the first block of the cued phase ($F_{1,26} = 4.21$, $p < 0.05$). Cage-reared hens also had a significantly higher latency to find the first bait than the aviary-reared hens for the uncued, cued and over-training phases ($F_{1,26} = 5.26$, $p < 0.03$; $F_{1,26} = 6.32$, $p < 0.02$; $F_{1,26} = 6.29$, $p < 0.02$). The same was observed for the transition between them (uncued-cued: $F_{1,26} = 6.19$, $p < 0.02$; cued-over-training: $F_{1,26} = 5.87$, $p < 0.03$). No significant treatment effects were found for the reversal phase. In conclusion, cage-reared hens were slower to find the first bait than aviary-reared hens and seemed to be more sensitive to changes in the environment, as shown by the differences during the transition between phases. Aviary-reared hens might therefore be better at adjusting to complex laying environments.

1. Introduction

In the egg industry, hens are usually kept in rearing farms before being transferred to the laying facilities. The type of environment experienced by the birds during the rearing period can differ greatly in degree of complexity. Indeed, in commercial production systems, chicks are usually reared in cage or aviary systems. The aviary system offers a much more complex environment, with among other things, the possibility for the chicks to dustbathe and to navigate in three dimensions by moving between the different tiers of the aviary. In both barren and furnished cage systems, chicks are confined to a smaller space where they can only access the tier they are housed in. After the rearing phase, pullets are transferred to laying facilities at 16–18 weeks of age where they are kept until 72–80 weeks of age. Since the ban on battery cages became effective in 2012 (Council of the European Union, 1999), birds in the EU are housed in furnished cages or alternative housing systems, such as barn, aviary or free-range. Partly due to welfare concerns from consumers and stakeholders, the industry is now moving towards cage-free housing systems. The shift from barren, less complex environments to environments presenting higher degrees of complexity demands more of the bird in terms of cognitive performance. Whether housed in a barn, aviary, or free-range systems, the birds must navigate their environment to find resources such as food, water, and nest boxes.

Rearing conditions are likely to affect cognitive abilities later in life.

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Early life is a critical period in development (Bateson et al., 2014; Di Segni et al., 2018): aversive experiences during early-life have long-lasting effects on the individual, including effects on cognitive abilities in mice and rats (Naninck et al., 2015; Alves et al., 2022). For laying hens, it means the environment experienced during rearing is an important factor influencing the development of the chicks (Janczak and Riber, 2015; Campbell et al., 2019). It has been shown that chicks which had no access to perches during early stages of life showed impaired spatial skills at the end of the rearing phase (Gunnarsson et al., 2000). It has also been shown that barren environments negatively affect spatial cognition in the short-term, up to seven weeks after transfer to the laying farm (Tahamtani et al., 2015). However, information is scarce on the longer-term effects of rearing in a barren environment.

Because early environmental complexity can influence cognition, we aimed at testing how two different rearing environments affect the cognitive abilities of the hens by using the spatial holeboard test (van der Staay et al., 2012). We focused on the medium-term effects of rearing on cognition and tested the hens between 32 and 40 weeks of age. The holeboard test is a task which has been used to assess different aspects of animal spatial cognition, such as learning and memory (van der Staay et al., 2012). It allows one to distinguish between working memory and reference memory. Working memory is a form of short-term memory, within a trial, whereas reference memory reflects long-term memory across trials. The spatial holeboard test has been used in several species, including in farm animals such as pigs (Arts et al., 2009; Roelofs et al., 2018), chickens (Nordquist et al., 2011; Ferreira et al., 2019) and more recently in calves (Lecorps et al., 2022). Compared to some other cognitive tests, such as the three-dimensional jump test, the holeboard test makes it possible to assess learning and cognitive abilities without the performances of the individuals being affected by their physical abilities.

To test the effect of early life environment on cognition, we reared hens either in a multi-tier aviary or in cages before transferring them to the laying farm at 18 weeks of age. The aviary representing a more complex environment, we expected hens reared in the aviary to show better cognitive abilities than hens reared in cages.

2. Material & methods

2.1. Animals, rearing and housing

2.1.1. Rearing

The hens used in this study (N = 64) were part of a larger project for which 384 non-beak trimmed White Leghorn hens were reared either in a cage (N = 192) or in an aviary (N = 192). They were then transported to an experimental farm at 18 weeks of age. The birds were reared at a commercial hatchery (Steinlands & co.) in one single room measuring 15 m x 72 m. The room contained 38000 birds housed in a raised NATURA Primus 16 system (Big Dutchman, www.bigdutchman.com, see Fig. 1). The system consisted of furnished cages measuring 12 m x 0.8 m x 0.6 m (length x height x width) stacked in three tiers. After hatching, chicks were placed on the first and second tier of the system. The mesh floor of the aviary rows was lined with paper until four weeks of age. Each aviary row was furnished with a feed line, nipple drinkers, and a perch above the water and feed lines. From 5 weeks of age, the front of the aviary rows was opened, and the birds could navigate between the different tiers and the floor of the house. They also had access to perches on the front of each tier of the aviary rows. The floor of the house was covered with wood shavings, and additional perches were extended from the front of each tier of the aviary rows at seven weeks of age (see Fig. 1). For one of the aviary rows, the front of one tier was kept closed during the whole rearing period. This enclosed space was located in the second tier of the aviary row and contained 250 birds. Thus, they had no access to the floor of the house or the other tiers of the aviary.

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. In the cage and aviary

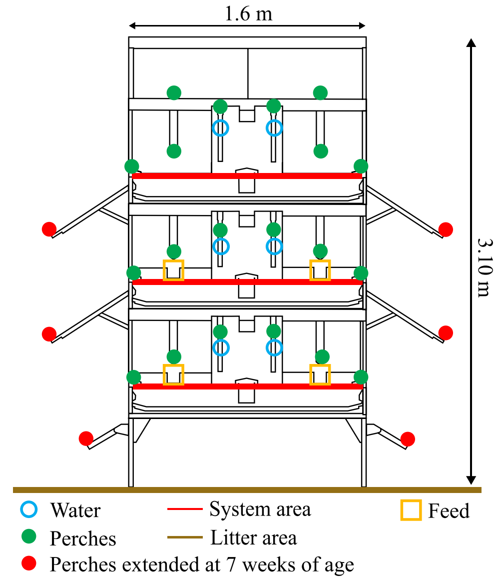

Fig. 1. Schematic representation of a raised Natura Primus 1600 viewed from the end of the row showing feed lines, water lines, and perches (based on the Big Dutchman leaflet).

conditions, birds had access to 9.6 cm and 3.2 cm of perch space per bird, respectively. 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 5 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 coccidiosis 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

At 18 weeks of age, the birds were transported to the experimental farm. The henhouse contained 2808 cages organised in 12 rows, each row containing six tiers. A walkway between the 3rd and 4th tier formed the second floor in the henhouse. Experimental birds were all housed in the third tier of the second floor, i.e., the top tier. They were housed in social groups of four individuals in two Victorsson T10 furnished cages adjoined by an opening (15 cm × 18 cm). The opening between the two cages allowed the birds to move freely between the two cages of the cage-pair. Each pair of cages containing four birds is hereafter referred to as a cage. Each cage measured 240 cm × 83 cm x 63 cm (width x height x depth) and the four birds sharing a cage came from the same rearing treatment. Each cage was furnished with four perches (75 cm perch space / bird), two nest boxes (1500 cm^2 each) and a dustbathing platform on the roof of each nest box (750 cm^2 / bird, Fig. 2). The

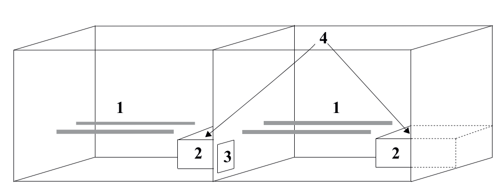

Fig. 2. Schematic representation of a furnished cage, three-quarter front view, showing (**1**) the perches, (**2**) the nest boxes, (**3**) the opening between the two parts of the cage and (**4**) the dustbathing trays.

treatments were distributed in the henhouse so that cages with birds reared in the aviary were next to cages with birds reared in cages. As part of another experiment, one bird per cage was removed at 24 weeks of age. The birds were thus housed in groups of three from that age on.

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.

2.2. Holeboard test

From 32–40 weeks, birds were tested in a holeboard test modified from Tahamtani et al. (2015) and Nordquist et al. (2011). It consisted of a habituation phase, followed by a training and testing phase. A pilot study previously led by our group showed that 33% of the birds did not consume any mealworms after several days of habituation. We, therefore, habituated 64 birds from 32 cages (16 cages with aviary-reared birds and 16 cages with cage-reared birds) to identify hens not engaging with the task. We then selected a subset of birds (one per cage) for the testing phases (see details in the habituation section).

As part of the habituation phase, additional data was collected and the birds were tested in a novel object test and an open field test. More details on the methods and results are available in Dumontier et al. (2022).

2.2.1. Testing arena

Two temporary arenas were built in the henhouse to test the birds. Each arena measured 350 cm × 177 cm x 190 cm (length x width x height). Three of the walls were made of wood frames covered with dark green tarps, the fourth wall being the concrete wall of the henhouse. Each arena was illuminated with a lamp fixed on one of the walls. Eight circles of 50 cm diameters were drawn with a marker on the floor (particle boards) of each arena. Circles were spaced 20 cm apart and were distributed in a 2 × 4 matrix (Fig. 3). A small pink cup designed for holding a single egg was glued onto a 19 cm × 19 cm plywood plate and the plate was placed in the centre of each circle drawn on the floor of the test arena. In each arena, a grey plastic box turned upside down was used as a start-box (40 cm × 30 cm x 20 cm, length x width x height). The start-box was randomly positioned on one of the short walls for each trial session and kept in the same position for all hens tested during the same trial session. To start the test the start-boxes were lifted by the experimenter from the outside of the arena using a rope attached to a pulley system. In this way it was possible to synchronize the start of the test in the two separate arenas in which birds were tested at the same time.

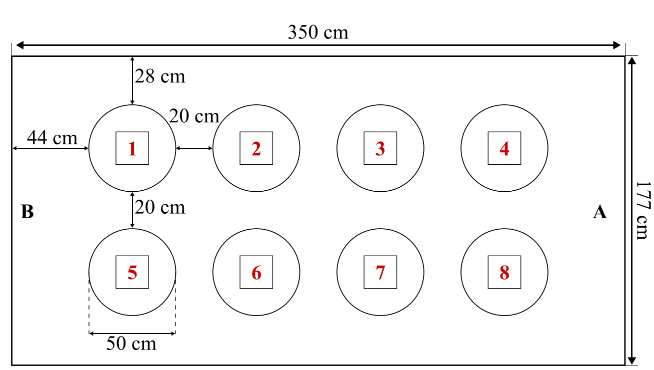

Fig. 3. Schematic representation of the holeboard arena, viewed from above. The letters A and B show the two possible positions of the start-box. Numbered squares represent the cups and plywood squares.

One camera (Axis m1124-e network camera, Noldus, The Netherlands) was mounted on the concrete wall of each arena at approximately 2.50 m to record the trials. All trials were recorded using the MediaRecorder system (Noldus Information Technology, Wageningen, The Netherlands).

2.2.2. Habituation phase

The habituation phase started when the hens were 32 weeks of age. They were habituated to the cups by three exposures per day for 5 days. During the first three days, they were presented with a small pink cup baited with three mealworms (known as a palatable food reward, Moe et al., 2009) directly in their cage. For the last two days of habituation, the cup was placed in the feed line. Each exposure to the baited cup lasted for 5 min, or until all mealworms were eaten.

Habituation to the arena was started when hens were 34 and 35 weeks of age. They were exposed daily for 5 days to the arena, each session lasting 5 min. During this habituation phase, all eight cups were baited with one mealworm to encourage exploration of the arena. For the first habituation session, two birds from the same cage were placed into the arena together to encourage them to explore. For the following sessions, they were exposed alone to the experimental setup. The number of mealworms eaten and the latency to eat the first mealworm were recorded for each habituation session. After habituation, the bird showing the best performance (number of mealworms eaten and latency to get the first reward) was selected for each cage. If no clear difference was observed between the two birds, one of them was randomly selected. Four cages (two with aviary-reared and two with cage-reared birds) were excluded because none of the hens consumed any mealworms. Thus, 14 cage-reared and 14 aviary-reared birds were included in the following holeboard test.

2.2.3. Training and testing phases

For all following phases, hens were trained/tested twice a day for 5 min except for the first day of the uncued phase where only one trial was performed. All tests were performed on workdays. Hens were always placed in the same arena and were returned to their cage between the two trials. The first cage tested was randomly chosen each day, and the order of testing (ascending or descending) was also randomly picked.

First, the birds were trained in an uncued phase for 12 days. During this phase, three cups out of eight were baited with one mealworm (see reward configuration section for more information). Then, the hens were trained in a cued configuration of reward for 4 days. The same configuration of baited cups as in the uncued phase was used, but the plywood squares under the baited cups were painted red (in place of the standard light brown colour) to give the hens additional cues. Following the cued phase, hens were trained for 4 days in an over-training phase. During this phase, baits were returned to their uncued form. Finally, hens were tested in a reversal phase for 5 days. During this phase, the hens were given a new configuration of uncued, baited cups.

2.2.4. Reward configuration

Across the 28 hens, 7 different configurations were used (4 hens per configuration, 2 cage- and 2 aviary-reared). Each configuration consisted of three mealworm-baited cups, and five empty cups. The configuration refers to the spatial position of the cup in the arena. The same configuration of baited cups was used during the uncued, cued and over-training phases. For the reversal phase, a new configuration of baited cups was randomly assigned to each bird.

2.2.5. Parameters recorded

For each trial, the latency to find the first bait (in seconds) and the trial duration (in seconds) were recorded. The trial duration was defined as the time elapsed between the start of the trial and the visit to the last baited cup or the maximum cut-off of 300 s, whichever occurred first. The total number of cups visited, the total number of visits to the baited set of cups and the number of different cups visited were recorded. These parameters were used to calculate the following variables:

- **Working Memory Ratio (WM):** Number of rewarded visits divided by the total number of visits to the baited cups. Shows the capacity to avoid revisiting baited cups that have already been visited.
- **General Working Memory Ratio (GWM):** Number of different cups visited divided by the total number of visits. Shows the capacity to avoid revisiting cups that have already been visited.
- **Reference Memory Ratio (RM):** Total number of visits to the baited cups divided by the total number of visits to all cups. Reflects the ability to discriminate between baited and unbaited cups.

WM and GWM are measures of short-term memory and are trial dependant, whereas RM gives a measure of long-term memory and is not trial dependant. For all memory ratios, scores close to 1 indicate good performances and scores close to 0 indicate poor performances in the holeboard test.

2.3. Data analysis

All statistical analyses were performed with R, version 4.2.1 (R Core Team, 2022). For the habituation phase ($N = 64$ hens), we used generalised linear mixed effects models on the number of worms eaten by each hen, using the package *glmmTMB* (Brooks et al., 2017). As the hens were exposed to the arena in pairs during the first day of habituation, it was excluded from the analysis. The rearing environment, the habituation day and whether the bird was selected for the holeboard test were used as categorical predictors. Two-way interaction between the predictors were also included. The Individual ID nested within the Cage ID was used as a random factor to account for repeated measures across days and lack of independence between birds from a same cage. The same model was also run separately on birds which were selected ($N = 28$) or not selected ($N = 36$) for the holeboard test to see any difference between rearing environments. P-values were calculated by Wald chi-square tests and models were checked for overdispersion and homogeneity of variances.

For the holeboard test ($N = 28$ hens), we used linear mixed effects models (LMMs) fitted by restricted maximum likelihood estimates, using the package *nlme* (Pinheiro et al., 2021). Trials during which the hen dustbathed before completing the task were excluded from the analysis (24 trials over the 1372 performed in total). In addition, 8 trials were excluded due to recording or baiting issues. The response variables were averaged across blocks of two consecutive testing days for the analysis. As the reversal phase lasted 5 days, the first two and last two days were averaged but only data from the third day was used to calculate the average for day 3. The rearing environment, trial blocks, and the two-way interaction were used as fixed effects. The interaction was removed when it was not significant, and the model was rerun after it was removed. Each phase was analysed separately. The bird ID was used as a random effect in the model to account for repeated measures. To study the effects of changes in the arena (addition/removal of cues, change in the baits configuration), the transition between each phase was also analysed. The same model as previously described for the different test phases was run on a subset of data containing only the last and first blocks of two consecutive phases. P values were calculated by F-tests. All models were checked for their conformation to the assumptions of parametric statistical models (homogeneity of variances and normal distribution of residuals). Time variables (latency to find the first bait and trial duration) were log transformed to make them fit these assumptions. The RM for the transition between the Uncued and Cued phase did not fulfil the assumptions and was therefore also log transformed.

3. Results

3.1. Habituation

Overall, cage-reared birds ate significantly fewer worms than hens reared in the aviary (Wald-$\chi 2$ (1) $= 10.62$, $p < 0.001$, see Fig. 4A, $N = 64$). The interaction between whether the hen was selected for the holeboard test or not and the habituation day was significant, with selected hens starting with a higher number of worms eaten (Wald-$\chi 2$ (3) $= 9.50$, $p = 0.02$, Fig. 4B, $N = 64$).

For both rearing environments, hens which were selected for the holeboard test ($N = 28$) did not differ in the number of worms eaten (Wald-$\chi 2$ (1) $= 0$, $p = 1$, Fig. 4B), and the number of worms they ate increased across habituation days (Wald-$\chi 2$ (3) $= 9.92$, $p < 0.02$). Looking at birds which were not selected for the holeboard test (Fig. 4B, $N = 36$), cage-reared hens ate significantly fewer worms than hens reared in the aviary (Wald-$\chi 2$ (1) $= 7.63$, $p < 0.01$). The number of worms eaten increased for hens from both rearing conditions across habituation days (Wald-$\chi 2$ (3) $= 61.77$, $p < 0.001$).

3.2. Holeboard test

Statistics from the holeboard test are summarised in Table 1. Data from the memory ratios are summarised in Fig. 5 and data from the time variables are summarised in Fig. 6.

3.2.1. Memory ratios

3.2.1.1. General working memory (GWM). The GWM ratio increased over time for cage- and aviary-reared hens during the Uncued phase ($F_{5135} = 7.12$, $p < 0.001$), the Cued phase ($F_{1,27} = 6.69$, $p < 0.015$) and the Reversal phase ($F_{2,52} = 6.39$, $p < 0.003$). The performance decreased during the transition between the Cued and Over-training phases ($F_{1,27} = 4.23$, $p < 0.05$, Fig. 5A), and between the Over-training and Reversal phases ($F_{1,27} = 11.02$, $p < 0.003$) for both treatment groups. No effects of the rearing environment were observed ($p > 0.05$, see Table 1).

3.2.1.2. Working memory (WM). The WM performances increased over time for both rearing environment during the Uncued and Cued phases ($F_{5135} = 4.63$, $p < 0.001$; $F_{1,27} = 6.78$, $p < 0.015$, respectively, Fig. 5B). No effects of the rearing environment were observed (see Table 1).

3.2.1.3. Reference memory (RM). For both cage- and aviary-reared hens, the RM ratio increased during the Uncued phase ($F_{5135} = 3.06$, $p < 0.012$) and the transition between the Uncued and Cued phases ($F_{1,27} = 27.95$, $p < 0.001$, Fig. 5C). The RM performances decreased during the transition between the Cued and Over-training phases ($F_{1,26} = 12.10$, $p < 0.002$) and between the Over-training and Reversal phases ($F_{1,27} = 13.87$, $p < 0.001$). The interaction between the rearing environment and the trial blocks was significant for the Cued phase ($F_{1,26} = 4.21$, $p < 0.05$), with hens reared in cages starting with a lower RM ratio than the aviary-reared hens but both groups reaching the same ratio at the end of the phase.

3.2.2. Time variables

3.2.2.1. Trial duration. Across time, hens from both rearing conditions became quicker at completing the task as shown by a decrease in trial duration during the Uncued phase, the Cued phase, and the Reversal phase ($p < 0.05$, see Table 1, Fig. 6A). The trial duration increased during all transitions between phases ($p < 0.05$, see Table 1). The cage-reared hens were slower than aviary-reared hens to complete the task during the transition between the Uncued and Cued phases ($F_{1,26} = 4.61$, $p < 0.02$).

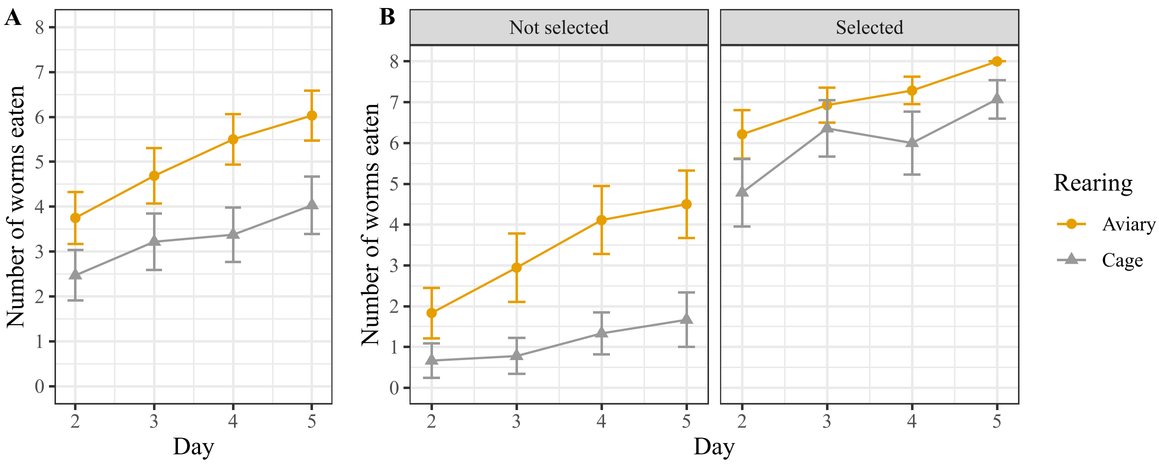

Fig. 4. Total number of worms eaten during the habituation phase (**A**) and number of worms eaten by the hens selected or not for the holeboard test (**B**) for each rearing environment. The graph shows the mean ± se.

Table 1
Results of linear mixed-effects models for all phases and transitions in the holeboard test. P-values were calculated by F tests.

	Rearing			Trial Block			Rearing x Trial Block		
	F	*df*	p≤	*F*	*df*	p≤	*F*	*df*	p≤
GWM									
Uncued	0.02	1,26	0.88	**7.12**	**5,135**	**< 0.0001**			n.s.
Trans. Uncued-Cued	0.18	1,26	0.68	0.47	1,27	0.50			n.s.
Cued	0.04	1,26	0.84	**6.69**	**1,27**	**0.02**			n.s.
Trans. Cued-Over Training	0.10	1,26	0.76	**4.23**	**1,27**	**0.05**			n.s.
Over Training	0.89	1,26	0.36	0.17	1,27	0.67			n.s.
Trans. Over Training-Reversal	0.00	1,26	0.98	**11.02**	**1,27**	**0.003**			n.s.
Reversal	2.04	1,26	0.17	**6.39**	**2,52**	**0.003**			n.s.
WM									
Uncued	1.07	1,26	0.31	**4.63**	**5,135**	**0.0006**			n.s.
Trans. Uncued-Cued	0.00	1,26	0.96	*3.86*	*1,27*	*0.06*			n.s.
Cued	0.20	1,26	0.66	**6.78**	**1,27**	**0.01**			n.s.
Trans. Cued-Over Training	0.12	1,26	0.73	1.61	1,27	0.22			n.s.
Over Training	0.11	1,26	0.74	0.00	1,27	0.99			n.s.
Trans. Over Training-Reversal	0.54	1,26	0.47	1.50	1,27	0.23			n.s.
Reversal	0.06	1,26	0.81	*2.45*	*2,52*	*0.10*			n.s.
RM									
Uncued	0.00	1,26	0.95	**3.06**	**5,135**	**0.01**			n.s.
Trans. Uncued-Cued	1.48	1,26	0.24	**27.95**	**1,27**	**< 0.0001**			n.s.
Cued	0.74	1,26	0.40	0.22	1,26	0.65	**4.21**	**1,26**	**0.05**
Trans. Cued-Over Training	0.53	1,26	0.47	**12.10**	**1,26**	**0.002**	*3.83*	*1,26*	*0.06*
Over Training	1.70	1,26	0.20	1.15	1,27	0.29			n.s.
Trans. Over Training-Reversal	0.00	1,26	0.98	**13.87**	**1,27**	**0.0009**			n.s.
Reversal	2.62	1,26	0.12	2.22	2,52	0.12			n.s.
Trial Duration									
Uncued	*2.94*	*1,26*	*0.10*	**27.45**	**5,135**	**< 0.0001**			n.s.
Trans. Uncued-Cued	**4.61**	**1,26**	**0.04**	**4.90**	**1,27**	**0.04**			n.s.
Cued	2.61	1,26	0.11	**36.15**	**1,27**	**< 0.0001**			n.s.
Trans. Cued-Over Training	0.72	1,26	0.40	**6.80**	**1,27**	**0.01**			n.s.
Over Training	0.41	1,26	0.53	0.02	1,27	0.90			n.s.
Trans. Over Training-Reversal	0.64	1,26	0.43	**10.88**	**1,27**	**0.003**			n.s.
Reversal	0.22	1,26	0.64	**4.10**	**2,52**	**0.02**			n.s.
Latency 1st bait									
Uncued	**5.26**	**1,26**	**0.03**	**8.21**	**5,130**	**< 0.0001**	*2.05*	*5,130*	*0.08*
Trans. Uncued-Cued	**6.19**	**1,26**	**0.02**	*4.04*	*1,27*	*0.05*			n.s.
Cued	**6.32**	**1,26**	**0.02**	**21.12**	**1,27**	**0.0001**			n.s.
Trans. Cued-Over Training	**5.87**	**1,26**	**0.02**	0.32	1,27	0.58			n.s.
Over Training	**6.29**	**1,26**	**0.02**	0.15	1,27	0.70			n.s.
Trans. Over Training-Reversal	2.39	1,26	0.13	**6.19**	**1,27**	**0.02**			n.s.
Reversal	0.48	1,26	0.49	2.25	2,52	0.12			n.s.

Significant comparisons ($p < 0.05$) are written in bold. Tendencies ($0.05 < p < 0.1$) are written in italics. GWM: General Working Memory; WM: Working Memory; RM: Reference Memory; Trans.: Transition

3.2.2.2. Latency to visit the first baited cup. Hens from both rearing conditions became faster at finding the first bait during the Uncued phase ($F_{5130} = 8.21$, $p < 0.001$) and the Cued phase ($F_{1,27} = 21.12$, $p < 0.001$, Fig. 6B). The latency to find the first bait increased during the transition between the Over-training and reversal phases ($F_{1,27} = 6.19$, $p < 0.02$). For the Uncued, Cued and Over-training phases and the transitions between them, aviary-reared hens were significantly faster to find the first bait than cage-reared hens ($p < 0.05$, see Table 1).

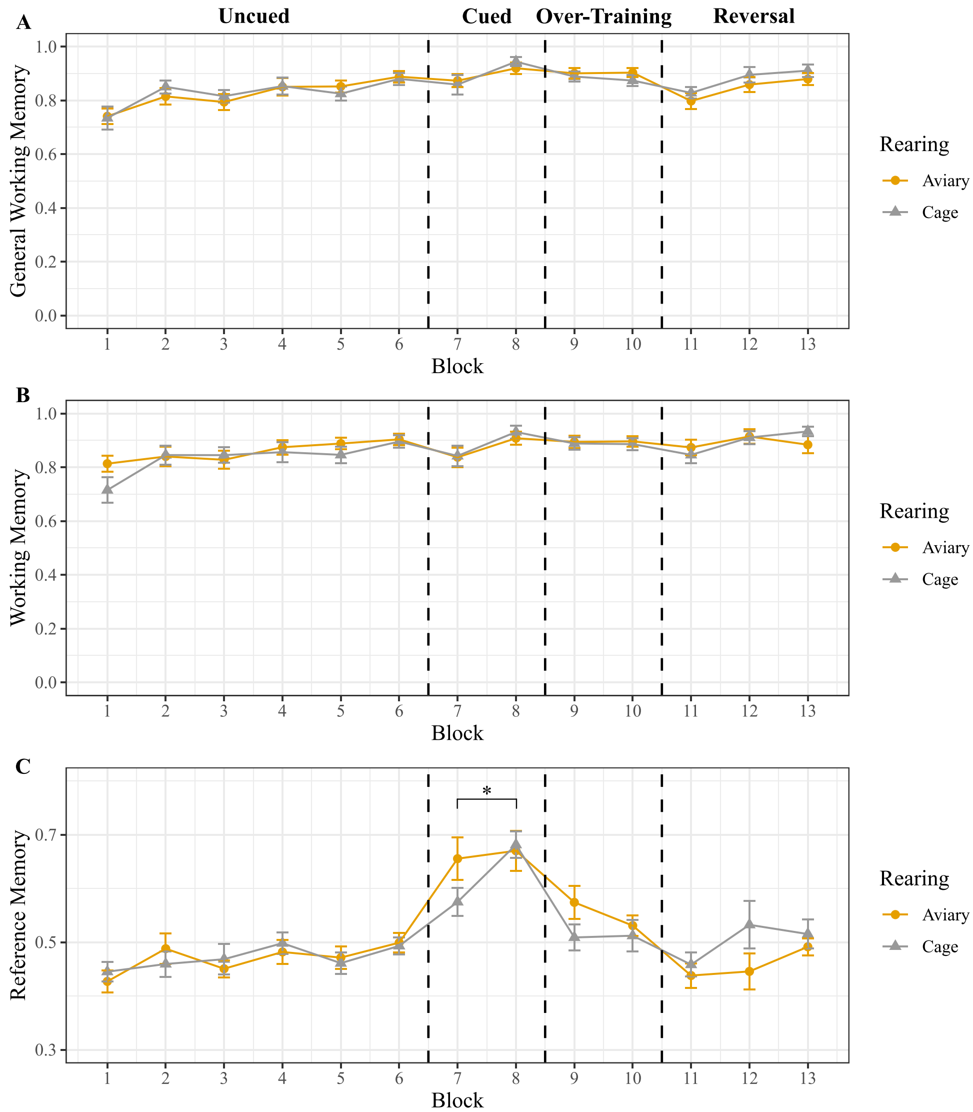

Fig. 5. Memory ratios from the holeboard test with the General Working Memory (**A**), the Working Memory (**B**) and the Reference Memory (**C**). A higher score indicates better memory performance. Trial blocks were averaged over two days of testing, except for block 12 which is the average of only the third testing day of the reversal phase (i.e., the average of two trials). The graphs show the mean ± se. * Indicates a significant interaction ($p < 0.05$).

4. Discussion

The aim of this study was to investigate whether the complexity of the rearing environment had medium-term effects on laying hens' spatial cognition. We predicted that due to a higher environmental complexity in the aviary, aviary-reared birds would show higher performances in the holeboard test compared to cage-reared birds.

4.1. Acquisition of the task

The results show an increase over time in general working memory (GWM) and working memory (WM) for both groups during the Uncued and Cued phases of the holeboard, reflecting that the birds revisited fewer cups with more experience of the task. This suggests that the birds got habituated to the arena and became more efficient at navigating it. This is supported by the fact that trial duration and the latency to the first baited cup also decreased over time, showing the birds were quicker to perform the task and to find the first bait. These results also support the idea that food deprivation prior to testing is not necessary for laying hens when the food reward is attractive (Arts et al., 2009; Nordquist et al., 2011; Tahamtani et al., 2015).

Previous studies on laying hens report WM ratios ranging between 0.7 and 0.8 (Nordquist et al., 2011; Tahamtani et al., 2015). The hens in our study demonstrated slightly higher WM ratios (0.8–0.9). This could be explained by differences in the study design. Indeed, Nordquist et al. (2011) and Tahamtani et al. (2015) tested the hens in a 3 × 3 matrix of cups, with three cups baited out of the nine. In the present study, we used a 2 × 4 matrix, with three cups baited out of the eight due to constraints in the space available to build the arenas in the henhouse. This design might be slightly simpler to navigate for the birds, which could explain the higher memory ratio.

Reference memory (RM) is usually used as an index to assess the ability of the individual to discriminate between baited and non-baited cups (van der Staay et al., 2012), and it reflects memory of the position of the baited cups across trials. In our study, RM ratios stayed relatively low (0.4–0.7) during all phases, though we observed an increase across time. These results are comparable to the ones obtained in previous studies on chickens (Nordquist et al., 2011; Tahamtani et al., 2015), but remained lower to the ones obtained in some studies on pigs (over 0.8 in Gieling et al., 2013; Grimberg-Henrici et al., 2016). It could be that birds did not learn the position of the baits and encountered them by chance while exploring the arena. Alternatively, it could be due to the fact that

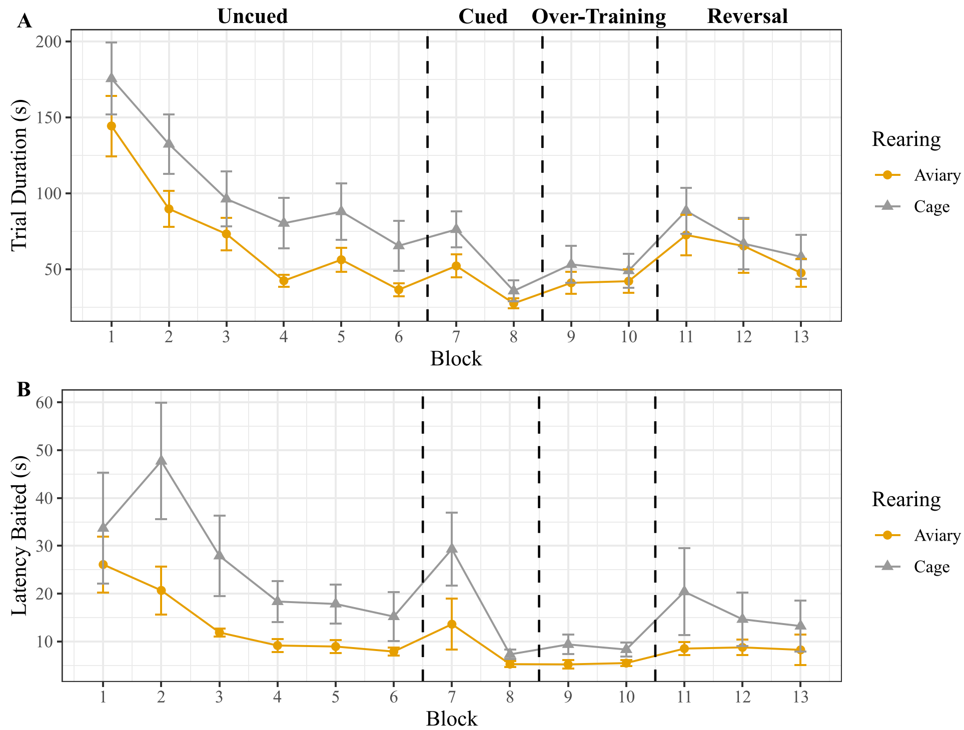

Fig. 6. Time variables from the holeboard test with the trial duration (**A**) and the latency to find the first bait (**B**). Trial blocks were averaged over two days of testing, except for block 12 which is the average of only the third testing day of the reversal phase (i.e., the average of two trials). The graphs show the mean ± se.

checking non-baited cups when moving from one baited cup to another is not costly. This second suggestion is supported by the drops in GWM and RM observed between the Over-Training and Reversal phases. Between those two phases, the configuration of baited cups of each bird is replaced by a new one, with no other changes in the arena. The GWM reflects the ability of the birds to avoid revisiting already visited cups (van der Staay et al., 2012). The drop in GWM between the Over-Training and Reversal phases thus indicates that the birds revisited cups more during the first block of the Reversal phase than during the last block of the Over-Training phase. It thus suggests that birds learned the position of the baits, at least to some extent. The drop in RM ratio suggests that birds revisited more unbaited cups during the Reversal phase than at the end of the Over-Training phase. They possibly revisit the locations of the baits from the previous configuration, but further analysis would be needed to confirm or invalidate this suggestion. To get clearer results on whether the birds discriminate between baited and non-baited cup, it could be interesting to increase the cost of visiting each cup. Indeed, White Leghorn hens have been shown to perform less contrafreeloading (i.e., choosing to forage over free food) than red jungle fowls (Jensen et al., 2002), and to get the majority of their food from the easily accessible site when offered the choice with a site requiring foraging (Schütz and Jensen, 2001). These findings suggest the hens might be more selective in their visits to the cups if they have to produce an effort to get access to the potential reward. This could be achieved, for example, by adding a swing lid to the cups so the hen has to dip its head inside the cup to check for food rewards.

4.2. *Effects of the rearing environment*

Overall, the rearing environmental complexity had few effects on the memory ratios measured during the holeboard test. This could be partly due to the fact that we selected the birds performing best at the end of the habituation phase to be tested in the holeboard task. To be able to assess the cognitive abilities of the birds, we had to select birds which were able to perform the task. In other words, birds which were actively exploring the arena and consuming rewards. The results from the habituation phase reflect that selection process, with birds which were selected for the holeboard test consuming more worms than birds which were not selected. Looking at the non-selected birds, there is a clear difference between the cage- and aviary-reared birds in the number of worms consumed. For each day of the habituation phase, cage-reared birds performed more poorly than aviary-reared birds and showed very low levels of worms eaten, despite five days of habituation. This could be either due to higher fear levels or lower cognitive abilities of the cage-reared birds. Previous research has demonstrated that increased environmental complexity during rearing reduced fearfulness in laying hens (Brantsæter et al., 2016; Nazar et al., 2022), which supports the idea that cage-reared hens might be more fearful. We saw that cage-reared birds started the Cued phase with a lower RM score than aviary reared birds but reached the same score by the end of the phase. This difference could reflect a stronger neophobic reaction to the introduction of cues (red plywood squares) for the cage-reared birds than for the aviary-reared birds. In contrast, results from our previous work showed that fear levels of birds reared in cages were comparable to the ones of aviary-reared birds when tested in an open field and a novel object test between 31 and 33 weeks of age (Dumontier et al., 2022). It seems that exposure to novelty alone in the arena (i.e., coloured cues) triggered a stronger neophobic reaction than the group exposure to a novel object in the cage (cup), and cage-reared hens seem to be more sensitive to changes in the environment than aviary-reared hens.

Tahamtani et al. (2015) reported a difference in working memory between hens reared in cages or in an aviary during the reversal phase. The results in our study do not present the same trend and the aviary- and cage-reared hens performed quite similarly. The hens tested in Tahamtani et al. (2015) were up to 23 weeks of age, whereas in our study they were up to 40 weeks of age. Those 17 additional weeks of housing in furnished cages at the laying farm could have evened out the effects of the rearing environment on cognition. A similar acclimatization to the laying environment has been observed by Pullin et al. (2020). Hens reared in barren cages showed a higher number of collisions than aviary-reared hens when transferred to an enriched colony, but the differences between the two groups dissipated by 49 weeks of age. This suggests that the rearing environment has initial effects on behaviour, but that the effects eventually fade over time. However, it is important to note that the study by Tahamtani et al. (2015) and ours differ on some aspects of the experimental design (matrix of cups, randomisation of the position of the start box) which also could have affected the performance of the hens.

Despite the effect of the selection process on the results, we still notice differences between the two treatment groups on the latency to find the first bait. This indicates a strong effect of the rearing environment as despite selecting the best birds to be tested, the two groups behaved differently. Hens reared in the aviary were faster to find the first bait than the hens reared in cages for the Uncued, Cued, Over-Training phases and the transition between them. However, no differences in the trial duration were found between the two groups, except for the transition between the Uncued and Cued phase. That difference in trial duration might be explained by the potentially stronger neophobic reaction of the cage-reared hens to the cues when first exposed to them, as previously discussed. The difference in latency to find the first bait might reflect a lower motivation to start the trial from the cage-reared hens, or a stronger reluctance to leave the start area and explore the arena.

5. Conclusion

Overall, the rearing environment had little effect on the cognitive performances of the hens selected to be tested in the holeboard task. Though few differences were observed between the groups, the results from the habituation phase show that cage-reared hens eat significantly fewer worms than aviary-reared hens. The selection process prior to testing might have evened out the potential differences between the two rearing environments. However, despite the effects of the selection process, cage-reared hens were slower to find the first bait than aviary-reared hens and seemed to be more sensitive to changes in the environment.

Ethical statement

The animals used in this study were enrolled in a larger project. An application for permission to perform the animal studies was 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 research animals (Forskrift om bruk av dyr i forsøk). Animals were always handled gently to minimize stress and only healthy animals were included in the study.

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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