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Welfare characteristics of laying hens in aviary and cage systems

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ABSTRACT

Concerns regarding the welfare of laying hens in cage systems (CS) have prompted the development of alternative housing systems, such as aviary systems (AS). However, debate remains about welfare and productivity under both systems. This study compares the welfare and egg quality of laying hens in CS and AS. Hy-Line Brown hens housed in CS (n = 79,500; cage space = 0.075 m^2/hen; 6.14 hens/cage) and AS (n = 42,079; floor space = 9 hens/m^2) on a single farm were evaluated at 28, 38, and 48 weeks of age. Five evaluators from the Welfare Quality® protocol were used to assess physical conditions (n = 600), and behaviors were assessed through qualitative behavior assessment (n = 15 flocks), avoidance distance test (ADT; n = 315), and novel object test (NOT; n = 15 flocks). Additionally, blood parameters (n = 50), egg quality (n = 50), and serum (n = 50) and egg yolk (n = 90) corticosterone levels were measured. Feather condition in CS hens deteriorated with age, showing higher plumage damage scores than AS hens, though both systems showed increases in comb pecking wounds and feather damage over time. The AS hens showed more positive behaviors, e.g., being active and energetic, while CS hens exhibited more negative behaviors, e.g., fearfulness and depression. In the AS, hens responded more quickly to the observer in the ADT ($P < 0.01$ for all periods), and more approached the object in the NOT ($P < 0.01$ at 38 and 48 weeks). The CS hens had higher yolk corticosterone levels ($P < 0.05$) at 48 weeks but produced heavier eggs consistently across all periods ($P < 0.01$ for all). In conclusion, this study highlights the importance of evaluating laying hen welfare through a combination of behavioral, physical, and physiological measures. Our findings suggest that the AS provides better welfare outcomes for hens than the CS, offering critical insights for improving both animal welfare and productivity in future housing systems.

Introduction

The poultry production industry is progressively phasing out cage systems (CS) due to increasing concerns regarding animal welfare (Kollenda et al., 2020). The absence of nests in CS is particularly detrimental to welfare (Duncan, 1992), as hens naturally prefer to lay eggs in nest boxes (Reed, 1994). Moreover, the CS limits natural behaviors such as roosting and scratching, affecting hens both physically and psychologically (Nicol, 1987; Baxter, 1994). Consequently, the industry has shifted its focus toward housing systems that better meet welfare standards (Broom, 2010; Butterworth, 2013; Sandøe et al., 2020).

Among the alternative systems, the aviary system (AS) has gained popularity among farmers due to its efficient use of space and ease of management compared to floor or free-range systems. The AS is a multi-tiered structure that offers an enriched environment, featuring a littered ground floor and multiple levels. This setup allows hens to engage in behaviors like running, wing flapping, and dustbathing, which are often restricted in cage settings (Leyendecker et al., 2005; Papageorgiou et al., 2023). Ahammed et al. (2014) suggest that AS can be a welfare-enhancing option for commercial farms, especially when considering the higher costs associated with animal welfare-certified eggs.

However, the AS also presents its own set of welfare challenges. For instance, non-beak-trimmed birds housed in AS may experience higher rates of feather damage and mortality (Vasdal et al., 2023). Furthermore, some studies suggest that AS may be less efficient than CS in terms of egg production (Philippe et al., 2020; Kato et al., 2022). Philippe et al. (2020) found that CS outperformed AS in egg-laying productivity as well

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as certain aspects of animal welfare. Despite these findings, the overall differences between the AS and the CS in both productivity and welfare remain unclear. Previous studies have demonstrated significant variations in egg quality (Cepero and Hernándiz, 2015), stress levels (Lay Jr et al., 2011), and feather-pecking behaviors (Heerkens et al., 2015) among housing systems. However, these variations could be influenced by factors such as experimental period, chicken breed, and beak trimming practices, as noted by Tahamtani et al. (2014), Blatchford et al. (2016), and Schwarzer et al. (2021). Therefore, to appropriately compare and evaluate the welfare and productivity of laying hens in AS and CS, it is necessary to control for these factors and conduct a comprehensive assessment that includes physical, behavioral, and physiological indicators.

This study compares the egg production performance and welfare indicators, encompassing physical condition scores, behavior, and stress hormone levels, of hens housed in cage and aviary systems. To minimize external variables, such as flock management and environmental conditions, the study was conducted on a single commercial farm that operates both CS and AS concurrently, with both systems adhering to Korean legal space requirements. We hypothesized that, when controlling for factors such as breed, nutrition, handling practices, and climatic conditions, laying hens will exhibit distinct production and welfare outcomes depending on their housing system. Furthermore, we expect this study to demonstrate that an approach combining physical condition evaluation and behavioral observation provides an effective means of assessing hen welfare and comparing it between housing systems.

Materials and methods

The study was conducted between February and August 2023 on a commercial farm located in eastern South Korea. The experimental procedure was reviewed and approved by the animal experimental ethics committee of Chonnam National University (approval number: CNU IACUC-YB-2023-05).

Birds, housing, and management

Hy-Line Brown laying hens, which were non-beak-trimmed, were reared in two different housing systems: (1) a cage system (CS) and (2) an aviary system (AS). For the CS, a total of 79,500 birds were housed at 16 weeks of age. Each hen had 0.075 m^2 of space, with an average of 6.14 hens per cage. The cages (as shown in Fig. 1A) were made of galvanized wire (62.5 cm × 60 cm × 60 cm) and equipped with one nipple drinker per cage, trough-type galvanized feeders providing 12 cm of space per hen. The CS used in this study is a battery cage system without a nest or perch, ensuring that hens were housed under conventional cage conditions rather than an enriched system. In the AS, a total of 42,079 birds were housed at 16 weeks of age with a stocking density of 9 hens/m^2. The aviary (as shown in Fig. 1B) had two tiers with nests enclosed by plastic curtains (0.5 m × 1.2 m). Each house was divided into two floors, with each floor containing three aviaries (14.41 m × 85.2 m). The total floor area was 2,457.2 m^2, with slat configurations spanning two tiers with three rows each, totaling 5,616.4 m^2 of usable space.

Feed was provided four times daily at 6:30 AM, 11:00 AM, 3:00 PM, and 5:30 PM, with ad libitum access to water. The poultry house temperature was automatically controlled according to the age of the hens and external temperatures, maintaining a range from 15°C to 22°C. Lighting was provided from 6:00 AM to 7:30 PM, followed by a dark period from 7:30 PM to 6:00 AM. This environment was considered thermoneutral and comfortable for the laying hens, in accordance with the Hy-Line Brown management guidelines (Hy-Line International, 2018).

Data collection

The physical condition and behavioral examinations of the laying hens were performed by five trained observers, with one observer assigned to each flock. Two of these observers were additionally responsible for collecting blood and egg samples. Prior to data collection, all observers practiced and standardized the scoring methods together. In the CS, evaluations were conducted on hens located in the top, middle, and bottom tiers of the cages (Fig. 1C). In the AS, evaluations were carried out in the aisles between the aviary structures (Fig. 1D). Sampling points in both systems were distributed across three locations on the first floor and two locations on the second floor.

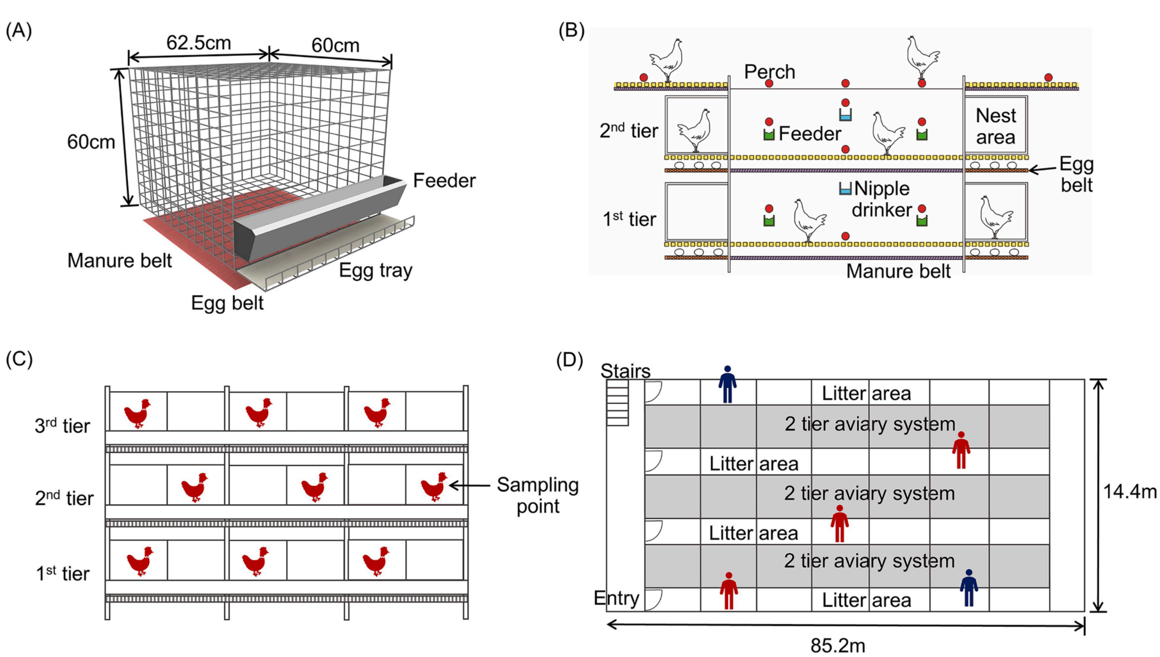

Fig. 1. Schematic diagrams of the housing systems and sampling points for laying hens in this study: (A) a cage, (B) an aviary, (C) a partial schematic of the cage system (CS) and sampling point for cages, and (D) views from the ceiling of the building showing 3 aviary structures (gray) with red-silhouettes representing sampling points for hens on the first floor, while blue-silhouettes representing those on the second floor.

Physical condition measurement

At 28, 38, and 48 weeks of age, three hundred hens from each housing system were assessed using a scoring system based on the welfare quality assessment (WQA) protocol for laying hens (Welfare Quality®, 2009). To ensure an unbiased selection process, 100 hens were evaluated at each week of age per housing system. Each evaluator randomly selected 20 hens per session, following a predefined protocol. The positioning of the evaluators during assessments is illustrated in Fig. 1(C, D). Comb wounds and foot pad lesions were scored, and the feather condition score was assessed on four parts of the body: the back of the head, neck, back, and breast.

Behavior assessments

All behavioral assessment procedures, which included qualitative behavior assessments (QBA), novel object tests (NOT), and avoidance distance tests (ADT) were conducted following the WQA protocol for laying hens (Welfare Quality®, 2009). All were conducted at 28, 38, and 48 weeks of age at the same locations where the physical condition assessments were conducted, ensuring consistency in observation environments. The positions of the evaluators during these assessments are illustrated in Fig. 1(C, D). The NOT was conducted four times per flock, with five evaluators assigned to different locations each week. The ADT was performed in three locations within each flock, with each evaluator conducting three assessments by slightly changing position within their designated area rather than staying in a fixed spot.

During each QBA, a flock was observed for 20 min in five different sections within each housing system. Hens were evaluated with 20 behavioral expressions using visual analog scales, which ranged from "0 = minimum" (absence of the behavior) to "10 = maximum" (prevalence of dominant expressions). The behavioral expressions included 10 positive emotions (active, relaxed, comfortable, confident, calm, content, energetic, friendly, positively occupied, and happy) and 10 negative emotions (fearful, agitated, depressed, tense, unsure, frustrated, bored, scared, nervous, and distressed).

For the NOT, the test object was a plastic pipe (30 cm in length and 2.5 cm in diameter) adorned with tape of different colors (black, red, white, green, and blue). The NOT was conducted four times per assessor, with a 5 min waiting period before placing the object. In AS, the object was placed on the floor, while in CS, it was placed on the feeder at chest height. The number of hens within 30 cm of the object was recorded every 10 s for 2 min, resulting in 12 counts per location. If more than 16 hens were within 30 cm, only 16 were recorded.

For the ADT, in AS, a hen situated 1.5 m from the assessor was observed. When the hen turned or retreated (as evidenced by a change in foot position), the distance from the assessor's hand to the position of the foot before the hen moved was measured. In CS, the assessor selected a cage with a hen's head protruding from the wire mesh and approached at a pace of one step per second until the hen withdrew into the cage. The distance from the evaluator to the cage was then measured.

Blood sampling and analysis

At 28, 38, and 48 weeks, 1 ml blood samples were collected from the brachial wing vein of 25 randomly selected hens and stored in a tube containing 0.5 ml of ethylenediaminetetraacetic acid (EDTA) for blood parameter analyses. A total of 75 hens were sampled across these three weeks for each treatment group. At 48 weeks, an additional 5 ml blood sample was collected from 25 randomly selected hens and stored in a 5 ml serum tube for corticosterone (CORT) analysis. At this time, blood from each hen was divided between the EDTA tube and the serum separator tube to allow for separate analyses of blood parameters and CORT levels.

On the day of sampling, cell counts were performed with a hematology analyzer (Exigo EOS, Boule Medical AB) using blood from the EDTA tubes, and the heterophil to lymphocyte (H:L) ratio was calculated. Blood samples in serum tubes were centrifuged at 3,000 rpm for 10 min, and the serum was decanted into new tubes and stored at −80°C until further analysis. Serum CORT concentrations were determined in duplicate using an ELISA kit (#ADI-901-097, Enzo Life Sciences, Farmingdale, NY, USA).

Egg quality measures and Corticosterone analysis in the yolk

One hundred eggs were collected from each housing system (both first and second floors) at 28, 38, and 48 weeks of age, and 25 of these (a total of 75 per housing system) were randomly selected each week for egg quality analysis. Among the remaining 25 eggs, 15 per week (a total of 45 per housing system) were randomly chosen for CORT analysis in the yolk. Egg collection was conducted after all behavioral assessments were completed. The following day, egg weights were measured with a precision of 0.01g using an analytical balance (ME104, Precision and analytical balances). Albumen weight was calculated as the difference between the egg weight and the combined weight of the yolk and shell. Haugh unit (HU) values were calculated using the formula $HU = 100 \times \log(H - 1.7 \times w^{0.37} + 7.6)$, where H is the height of the albumen (mm) and w is the egg weight (g), as outlined by Eisen et al. (1962).

After measuring egg quality, the yolk samples from 15 randomly chosen eggs were stored at −80°C for CORT analysis. The CORT extraction from the egg yolk followed the method described by Abobaker et al. (2017). The analysis was performed in duplicate using commercial CORT ELISA kits (#ADI-901-097, Enzo Life Sciences, Farmingdale, NY, USA).

Statistical analysis

The statistical analysis of the data was performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, United States). The normality of the data was examined using the Shapiro–Wilk test in the UNIVARIATE procedure. Physical condition measurements, NOT, ADT, H:L ratios, blood parameters, and CORT levels in the egg yolks were analyzed using the PROC GLIMMIX with a lognormal distribution. In this model, housing system (cage vs. aviary), age (28, 38, and 48 weeks), and their interaction were included as fixed effects, while flock was treated as a random effect to account for variability within groups. The PROC MIXED model was applied to analyze serum CORT and egg quality data. A principal component analysis (PCA) was employed for the QBA data. A P-value of < 0.05 was considered significant, while P-values between 0.05 and 0.10 were considered indicative of a tendency. All possible correlations between behavioral measures, blood characteristics (including CORT levels), and plumage condition were analyzed using Spearman's rank correlation, and only the significant correlations are reported in the results.

Results

Mortality and productivity

In CS, the overall accumulated mortality rates for the entire housing system were 1.3 % at 28 weeks, 3.3 % at 38 weeks, and 6.5 % at 48 weeks of age. In contrast, the AS showed higher overall accumulated mortality rates, with 2.6 % at 28 weeks, 5.6 % at 38 weeks, and 8.7 % at 48 weeks of age.

Regarding laying rates, hens in CS showed consistent performance, with rates of 89.0 % at both 28 and 38 weeks, and 88.0 % at 48 weeks. In comparison, hens in AS initially exhibited a higher laying rate of 93.8 % at 28 weeks, which slightly decreased to 89.2 % at 38 weeks but then increased again to 92.3 % at 48 weeks.

Physical condition score

In all parameters except comb pecking wounds and plumage damage on the back, CS hens showed higher scores than AS hens, indicating that the feather condition of the hens in CS was poorer ($P < 0.05$ for all, Table 1). In comb pecking wounds, the AS produced a higher score than the CS at 28 weeks ($P = 0.001$), but there was no difference thereafter (Table 1). For plumage damage on the back, the CS hens had a higher score than AS hens at 28 weeks ($P = 0.01$) and a tendency toward a higher score appeared at 48 weeks ($P = 0.069$), but there was no difference at 38 weeks ($P > 0.05$) (Table 1). In CS, damage to the feathers on the back of the head, neck, back, and breast increased with age ($P < 0.05$ for all, Table 1). In AS, the incidence of injuries such as comb pecking wounds and damage to the back of the head, neck, and back also escalated with age ($P < 0.05$ for all, Table 1).

Behavioral observations

In the QBA, the first two components of the PCA accounted for 71.5 % of the behavioral variance in CS and 69.4 % in AS. Component 1 reflects the negative emotional valence of laying hens, with behaviors ranging from scared and bored in CS (Fig. 2A) to depressed and fearful in AS (Fig. 2B). Component 2 explains the positive emotional valence, ranging from energetic and content in CS (Fig. 2A) to calm and friendly in AS (Fig. 2B).

In the ADT, the AS group scored significantly higher than the CS group at 28, 38, and 48 weeks of age ($P < 0.01$ for all, Fig. 3), indicating that the hens in the AS group were quicker to avoid the observer when approached. The ADT score was negatively correlated with CORT levels in the yolk ($r = -0.613$; $P < 0.01$). In the NOT, AS laying hens exhibited a greater willingness to approach novel objects compared to the CS hens at 38 and 48 weeks ($P < 0.01$, for both), while no difference was observed at 28 weeks (Fig. 4). Additionally, a negative correlation was found between NOT scores and the plumage condition of the breast ($r = -0.736$; $P < 0.05$) indicating that laying hens with breast plumage damage were more reticent to approach unfamiliar objects.

Blood characteristics and serum Corticosterone concentrations

Hens in AS showed higher H:L ratios than those in CS at 48 weeks ($P < 0.01$), while no significant differences were observed at 28 and 38 weeks of age (Fig. 5). White blood cell (WBC) counts showed no significant difference between CS and AS hens at 28 and 38 weeks, but WBC counts were higher in AS hens at 48 weeks of age ($P = 0.003$, Table 2). Additionally, monocyte and eosinophil counts were higher in the AS group, with significant differences observed at 48 weeks ($P = 0.002$, $P = 0.001$, respectively; Table 2). Significant differences were also noted for red blood cell counts at 28 weeks ($P = 0.015$), neutrophil counts at 48 weeks ($P = 0.001$), and hemoglobin concentrations at 28 weeks ($P = 0.016$) (Table 2). Differences in total platelet counts approached significance overall ($P = 0.050$), with notable differences observed at 28 weeks ($P = 0.003$, Table 2). However, lymphocyte counts and serum CORT concentrations did not significantly differ between the two housing systems (Table 2).

Egg quality and yolk corticosterone concentrations

Overall, eggs from the CS were heavier compared to those from the AS ($P = 0.001$), and this trend was consistent at 28, 38, and 48 weeks ($P = 0.001$ for all, Table 3). Similarly, the average yolk weight ($P = 0.001$) and overall albumen ($P = 0.001$) and shell ($P = 0.011$) weights were higher in the CS laying hens (Table 3). The HU showed no significant difference between the housing systems (Table 3).

At 28 and 38 weeks of age, there was no significant difference in egg-yolk CORT levels between the eggs produced by CS and AS hens (Fig. 6). However, at 48 weeks, the CS hens produced eggs with higher levels of CORT in the yolks ($P < 0.05$, Fig. 6).

Table 1
Physical condition scores of laying hens housed in cage (CS; $n = 300$ hens) and aviary (AS; $n = 300$ hens) systems at 28, 38, and 48 weeks of age.

Parameters	Age (wks)	Housing type		SEM	*P*-value		
		CS[1]	AS[1]		Housing	Age	Housing × Age
Comb pecking wounds[2]	28	0.1	0.6[a]	0.04	0.001	0.002	0.001
	38	0.2	0.3[b]	0.03	0.217		
	48	0.2	0.2[b]	0.04	0.192		
	Mean	0.2	0.4	0.02	0.001		
Plumage damage on the back of head[3]	28	1.1[b]	0.3[b]	0.06	0.001	0.001	0.015
	38	1.6[a]	1.1[a]	0.06	0.001		
	48	1.6[a]	1.1[a]	0.05	0.001		
	Mean	1.4	0.8	0.04	0.001		
Plumage damage on the neck[4]	28	0.7[b]	0.2[c]	0.05	0.001	0.001	0.004
	38	1.7[a]	1.0[b]	0.05	0.001		
	48	1.8[a]	1.5[a]	0.04	0.001		
	Mean	1.4	0.9	0.03	0.001		
Plumage damage on the back[4]	28	0.2[c]	0.1[c]	0.03	0.010	0.001	0.839
	38	0.9[b]	0.8[b]	0.06	0.249		
	48	1.5[a]	1.3[a]	0.05	0.069		
	Mean	0.9	0.7	0.04	0.010		
Plumage damage on the breast[4]	28	0.1[c]	0.0	0.02	0.004	0.001	0.001
	38	1.1[b]	0.6	0.06	0.001		
	48	1.5[a]	0.5	0.06	0.001		
	Mean	0.9	0.4	0.03	0.001		
Foot pad lesions[5]	28	0.2	0.1	0.02	0.011	0.720	0.476
	38	0.2	0.0	0.02	0.001		
	48	0.2	0.0	0.02	0.001		
	Mean	0.2	0.0	0.01	0.001		

[1] Superscript letters ([a])
[b], and [c]) indicate significant differences across ages ($P < 0.05$).
[2] 0 = none, 1 = less than 3 pecking wounds, 2 = 3 or more pecking wounds
[3] 0 = no or slight wear, 1 = one or more featherless areas < 2.5 cm, 2 = naked patch ≥ 2.5 cm.
[4] 0 = no or slight wear, 1 = one or more featherless areas < 5 cm, 2 = naked patch ≥ 5 cm.
[5] 0 = intact, no wounds, 1 = necrosis or proliferation of epithelium, 2 = swollen dorsally visible.

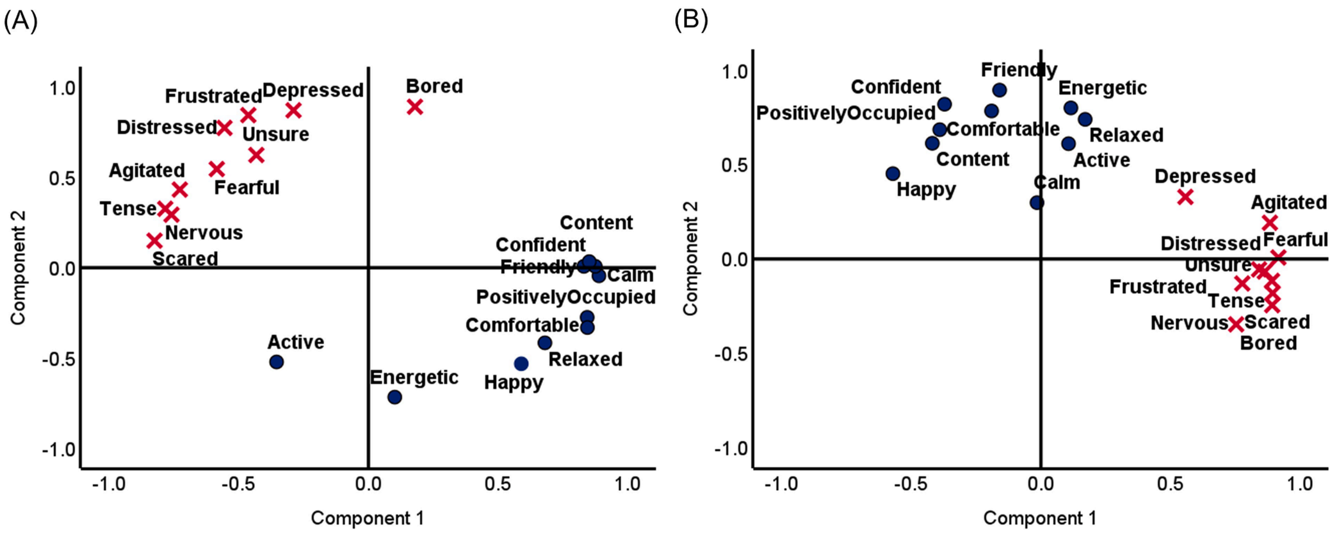

Fig. 2. Qualitative behavior assessment (QBA) results for laying hens housed in cage (A; $n = 15$ flocks) and aviary (B; $n = 15$ flocks) systems. A total of the 20 descriptors were evaluated, including 10 positive (blue dots; active, relaxed, comfortable, confident, calm, content, energetic, friendly, positively occupied, and happy) and 10 negative (red Xs; fearful, agitated, depressed, tense, unsure, frustrated, bored, scared, nervous, and distressed) terms.

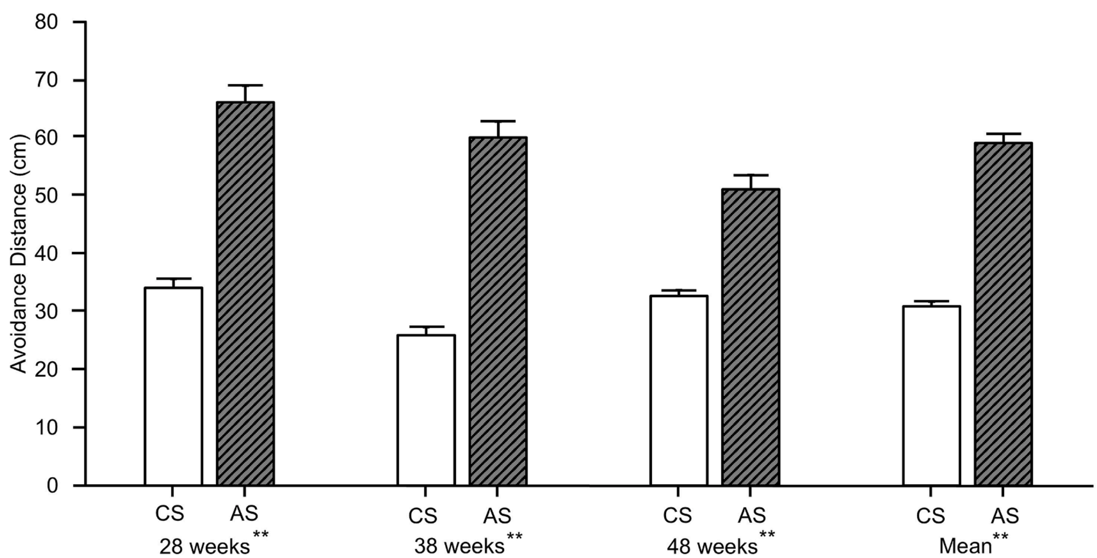

Fig. 3. Avoidance distance test (ADT) for laying hens in cage (CS; $n = 315$ hens) and aviary (AS; $n = 315$ hens) systems. Data are shown as means ± SEM. Asterisks indicate that means differ between the CS and AS (**, $P < 0.01$).****

Discussion

This study provides valuable insights into the welfare and productivity of laying hens housed in different systems, specifically comparing cage and aviary systems. Notably, hens in the AS consistently exhibited better feather conditions on their back and wings compared to those in the CS at all ages. The AS hens also exhibited greater movement, as indicated by higher ADT values, which likely reflects their ability to move freely rather than merely heightened fear. Their more frequent interactions with novel objects in the NOT suggest increased curiosity and potentially lower fear responses. These behaviors, along with lower yolk CORT levels, suggest that AS provide a more enriched and welfare-friendly environment. In contrast, CS hens, while producing heavier eggs, exhibited more negative behaviors and had higher yolk CORT levels. These findings emphasize the need to balance productivity with welfare considerations in housing systems and improve housing environments to promote both animal welfare and egg production. Further research should focus on additional factors influencing welfare outcomes within various housing systems, with the goal of further optimizing welfare strategies and enhancing the overall well-being of laying hens.

Plumage condition is a key indicator of hen health, reflecting the effects of their interactions with both the housing environment and other birds (Pichová and Bilčík, 2017). Hence, assessing plumage conditions offers valuable insights into the welfare status of laying hens across various housing systems. Previous research has indicated that feather pecking, including behaviors such as cannibalistic attacks and pecking that damage skin and tissue, is associated with stress, discomfort, and fear (Bilč ík and Keeling, 1999; Gunnarsson, 1999; Lambton et al., 2013). This could explain our current findings, where the higher scores observed in all feather pecking parameters in the CS suggest increased stress levels in the hens. This is in line with previous studies by de Haas et al. (2013) and El-Lethey et al. (2000). It is plausible to speculate that these elevated scores may be attributed to the stressful conditions present within the housing environments. Several authors have observed that housing systems affect plumage conditions (Appleby

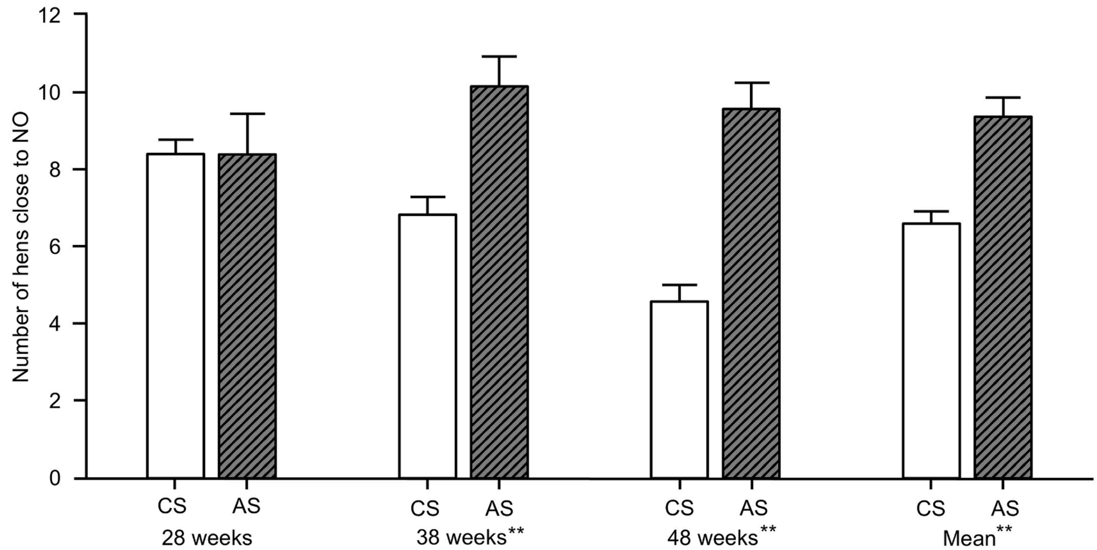

Fig. 4. Novel object test (NOT) scores for laying hens housed in cage (CS; $n = 15$ flocks) and aviary (AS; $n = 15$ flocks) systems. Data are shown as means ± SEM. Asterisks indicate that means differ between the CS and AS (**, $P < 0.01$).

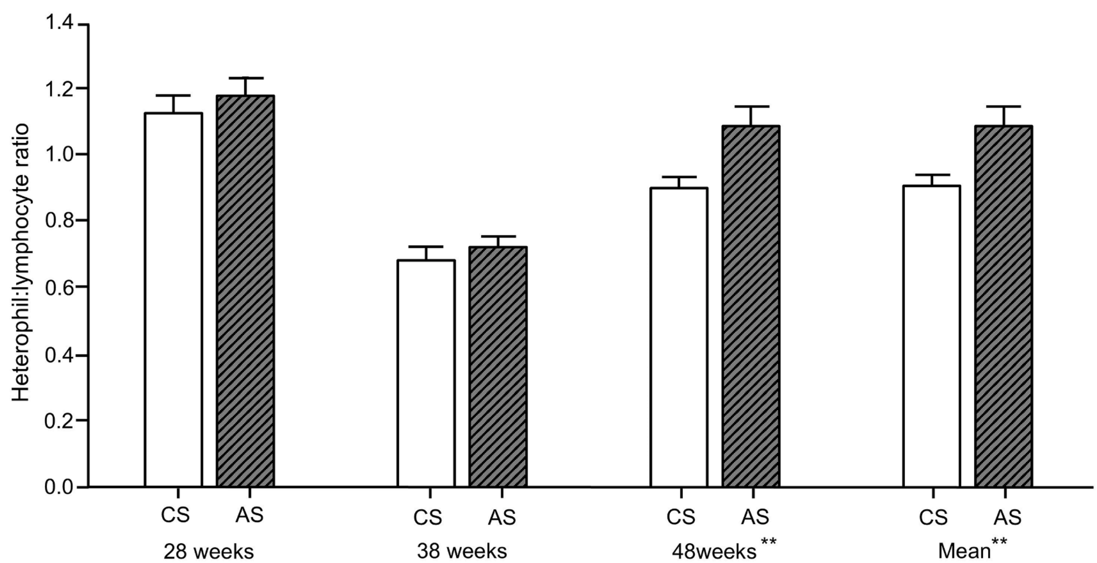

Fig. 5. The heterophil-lymphocyte (H:L) ratio for 28-, 38-, and 48-week-old laying hens housed in cage (CS; $n = 75$) and aviary (AS; $n = 75$) systems. Data are shown as means ± SEM. Asterisks indicate that means differ between the CS and AS (**, $P < 0.01$).

et al., 2002; Weitzenbürger et al., 2006), while others found no evidence of such impacts (Moinard et al., 1998). Differences in feather pecking scores were evident as early as 28 weeks of age in the present study, suggesting that the disparity in stress levels may have begun early in the rearing period, possibly starting from the pullet stage, rather than between 28 and 48 weeks. The lack of change in feather pecking scores between 38 and 48 weeks suggests that the hens may have already experienced significant feather damage by 38 weeks and that feather pecking behavior stabilized thereafter. In several studies, the condition of the hens' plumage deteriorated with increasing age (Hinrichsen et al., 2016; Sokołowicz et al., 2023). Such deterioration could be due to plumage wear, the accumulation of damage, or an increase in severe feather pecking behavior, but most damage has been confirmed to be caused by feather pecking (Bilč ík and Keeling, 1999). These findings highlight the importance of early intervention during the rearing process to manage stress and prevent severe feather damage over time.

Evaluating comb wounds in laying hens is commonly used to assess aggression, often interpreted as a response to stress (Roenchen et al., 2007). However, we found a higher incidence of comb pecking wounds in the AS group, suggesting they may result from the dynamics of increased space and social interactions. In AS, hens have more freedom to move, which could lead to more frequent comb pecking due to heightened opportunities for social interaction (Engel et al., 2019; Tünaydın and Dikmen, 2019). In contrast, hens housed in CS likely could only rarely engage in comb pecking behavior due to the physical limitations on their movement within the confined space. However, Elson and Croxall (2006) observed a higher incidence of comb injuries in CS, indicating that aggression can also occur in more restricted environments. Our study found that despite the increased frequency of comb pecking in AS, there were no severe injuries over the 48-week period. Direct behavioral observations were not conducted to confirm whether comb pecking was primarily associated with dominance interactions, so our interpretation remains hypothetical. However, the absence of severe injuries suggests that, in AS, pecking may function as a low-intensity social interaction rather than a form of harmful aggression. This suggests that while the AS environment allows for more natural behaviors,

Table 2
Blood parameters of laying hens housed in cage (CS; $n = 75$ hens) and aviary (AS; $n = 75$ hens) systems at 28, 38, and 48 weeks of age.

Parameters	Age (wks)	Housing type		SEM	*P*-value		
		CS[1]	AS[1]		Housing	Age	Housing × Age
White blood cell (K/μL)	28	31.80^{a}	28.29^{a}	1.209	0.914	0.001	0.253
	38	21.65^{b}	23.94^{b}	0.867	0.169		
	48	25.95^{b}	26.90^{a}	0.608	0.003		
	Mean	25.92	26.90	0.593	0.354		
Lymphocyte (K/μL)	28	11.42	10.39	0.296	0.221	0.075	0.583
	38	9.86	10.55	0.287	0.180		
	48	10.39	10.16	0.165	1.000		
	Mean	10.35	10.16	0.161	0.574		
Monocyte (K/μL)	28	6.71^{a}	6.58	0.182	0.643	0.001	0.312
	38	4.87^{b}	5.31	0.172	0.244		
	48	5.65^{ab}	6.02	0.108	0.002		
	Mean	5.66	6.02	0.106	0.048		
Neutrophil(K/μL)	28	13.27^{a}	12.07^{a}	0.621	0.935	0.001	0.146
	38	6.84^{c}	7.91^{b}	0.429	0.342		
	48	9.66^{b}	10.75^{a}	0.351	0.001		
	Mean	9.67	10.75	0.343	0.064		
Eosinophil (K/μL)	28	0.39	0.36	0.045	1.000	0.001	0.084
	38	0.06	0.16	0.021	0.060		
	48	0.23	0.34	0.025	0.001		
	Mean	0.22	0.34	0.024	0.003		
Hemoglobin concentration (g/dL)	28	15.56	14.65	0.213	0.016	0.135	0.008
	38	14.61	14.68	0.310	0.393		
	48	14.80	14.88	0.158	0.303		
	Mean	0.22	0.34	0.024	0.967		
Red blood cell (K/μL)	28	2.49	2.30	0.034	0.015	0.034	0.123
	38	2.32	2.36	0.050	0.829		
	48	2.35	2.34	0.025	0.850		
	Mean	2.36	2.34	0.025	0.605		
Total platelet count (K/μL)	28	17.04^{a}	7.60	1.837	0.003	0.010	0.036
	38	6.92^{b}	7.64	0.355	0.355		
	48	9.48^{c}	7.07	0.692	0.065		
	Mean	9.52	7.07	0.674	0.050		
Serum corticosterone (pg/ml)	48	5737.54	8187.90	1588.879	0.446		

[1] Superscript letters (^{a}, ^{b}, and ^{c}) indicate significant differences across ages ($P < 0.05$).

Table 3
Quality of eggs from laying hens housed in cage (CS; $n = 75$ eggs) and aviary (AS; $n = 75$ eggs) systems at 28, 38, and 48 weeks of age.

Parameters	Age	Housing type		SEM	*P*-value		
		CS[1]	AS[1]		Housing	Age	Housing × Age
Egg weight (g)	28	60.98^{b}	57.79	0.508	0.001	0.001	0.301
	38	63.76^{ab}	58.57	0.695	0.001		
	48	65.07^{a}	59.59	0.735	0.001		
	Mean	63.27	58.65	0.515	0.001		
Yolk weight (g)	28	14.67	13.19^{b}	0.178	0.001	0.001	0.131
	38	15.46	13.94^{a}	0.182	0.001		
	48	15.21	14.46^{a}	0.164	0.020		
	Mean	15.11	13.87	0.136	0.001		
Albumen weight (g)	28	39.57^{b}	38.20	0.410	0.060	0.010	0.040
	38	41.55^{ab}	38.21	0.531	0.001		
	48	43.14^{a}	38.54	0.587	0.001		
	Mean	41.42	38.32	0.407	0.001		
Shell weight (g)	28	6.75	6.40	0.120	0.050	0.703	0.528
	38	6.75	6.42	0.070	0.015		
	48	6.72	6.59	0.073	0.378		
	Mean	6.74	6.47	0.059	0.011		
Haught unit	28	92.62	92.87	0.134	0.795	0.797	0.867
	38	92.78	91.78	0.697	0.479		
	48	92.11	91.80	1.141	0.890		
	Mean	92.50	92.15	0.670	0.708		

[1] Superscript letters (^{a}, ^{b}, and ^{c}) indicate significant differences across ages ($P < 0.05$).

including pecking as a form of social interaction, the behavior might be less intense or more regulated, possibly due to the larger space and a more complex social structure. Therefore, the presence of comb wounds should not be interpreted solely as a sign of aggression or poor welfare but rather as a natural behavior related to social hierarchy. Further research is needed to directly assess the role of comb pecking in social dynamics and its implications for welfare, as well as to develop effective strategies to manage this behavior.

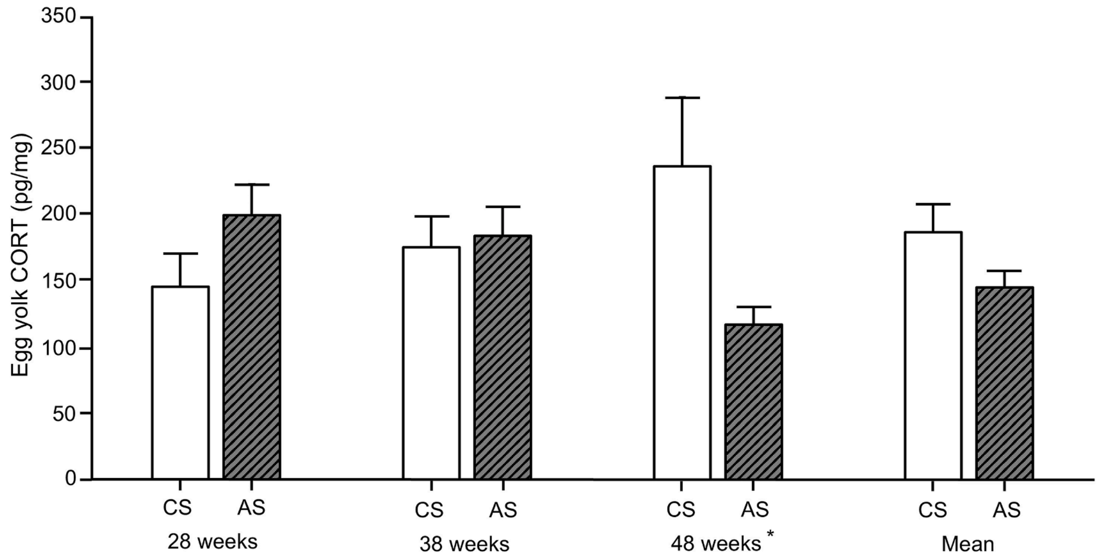

Fig. 6. Egg yolk corticosterone (CORT) concentrations for laying hens housed in cage (CS; n = 45 eggs) and aviary (AS; n = 45 eggs) systems. Data are shown as means ± SEM. Asterisks indicate that means differ between the CS and AS (*, $P < 0.05$).

The QBA evaluates both negative and positive aspects of animal experiences, focusing on their affective states and behavioral expressions (Fleming et al., 2016). Hence, it provides insight into animals' emotional and behavioral states, which makes it an effective tool for assessing animal welfare (Wemelsfelder and Lawrence, 2001). In the present study, CS hens exhibited behaviors indicative of stress, fear, and boredom more frequently, whereas AS hens demonstrated behaviors associated with happiness and activity more often. These findings are consistent with previous studies (Shimmura et al., 2010; Nenadović et al., 2022) comparing CS and AS. Lay Jr et al. (2011) suggested that the behavioral restrictions in CS can lead to increases in negative behaviors and decreases in positive behaviors. The greater expression of positive behaviors observed in AS hens in this study may be attributed to their increased opportunities for natural behaviors and a wider available range of movement.

Similarly, Niekirk et al. (2012) found that QBA scores were lowest in conventional cages and highest in AS, likely due to limited space for behavioral expression in cages and the potential influence of the observer effect. In our study, we analyze the QBA data using PCA, which is commonly used to reduce dimensionality and highlight key behavioral patterns that contribute to overall welfare scores. This enabled us to group related behavioral expressions and identify primary factors driving the differences in QBA scores between housing systems. The PCA-derived scores in our study suggest that, while the QBA effectively captures the behavioral expressions and emotional valence of the hens, it does not directly correlate with their physical condition. This aligns with the findings of Vasdal et al. (2022), who also found no direct relationship between QBA scores and physical condition. The lack of this correlation suggests that, while QBA are effective for assessing behavioral and emotional states, they may not fully reflect the physical health of animals. However, it is important to acknowledge that QBA relies on subjective assessment, and the observer's awareness of housing conditions could introduce potential bias. Further research incorporating blinded assessments or automated behavioral analysis is necessary to better understand the relationship between behavioral assessments and physical welfare indicators in laying hens.

The NOT assesses the competing motivations of animals to either approach or avoid new objects (Miller, 1944), and it is widely employed to gauge levels of fearfulness in poultry (Jones, 1996). We found that, on average, 2.8 fewer laying hens approached a novel object in the CS (6.6 hens) than in the AS (9.4 hens). The placement of the novel object in the CS, which was in front of the feed trough, might have reduced its visibility to the hens inside the cages, limiting the number of hens that approached it. Additionally, the present results showed a tendency for fewer CS hens to gather around the novel object as they aged, which may indicate a reduction in environmental stimulation or a decrease in their ability to exhibit natural behaviors as they grow older. In contrast, AS hens consistently showed a willingness to gather around the novel object, with significantly more responding to the object in the AS than in the CS at 38 and 48 weeks of age. This supports a previous finding that the AS better fosters hens' natural behaviors, with reduced fearfulness and increased exploration linked to superior physical health (Nannoni et al., 2022). This is further evidenced by the correlation observed in this study between the extent to which the laying hens approached novel objects and their breast plumage condition. A recent study has demonstrated that flocks experiencing feather damage and cannibalism exhibit greater fear of novel objects (Hüttner et al., 2023). Indeed, an increased fear response in hens has been associated with feather pecking (Rodenburg and Koene, 2004; Uitdehaag et al., 2008), which in turn can cause feather damage and cannibalism (Rodenburg et al., 2013). However, it is also possible that hens experiencing feather pecking become more fearful as a result of this behavior, suggesting a bidirectional relationship. As numerous studies have suggested, the results of this study further confirm that the NOT is a suitable method for assessing fear responses in laying hens (Uitdehaag et al., 2008; Rozempolska-Rucinska et al., 2017; Tahamtani et al., 2023; Hüttner et al., 2023).

Avoidance behavior in hens serves as a critical indicator of their fearfulness towards humans (Graml et al., 2008). The observed differences in avoidance behavior between hens in CS and AS suggest varying levels of habituation to humans and comfort around them, likely influenced by the specific environmental conditions of each housing system. These findings are consistent with those of Graml et al. (2008), which compared cage and free-range systems and revealed a similar pattern, indicating a consistent trend across various housing environments. The greater avoidance distances observed in AS hens may indicate increased stress and social anxiety, making these hens more likely to evade close human contact. However, it is important to note that the AS offers a more intricate environment than the CS, with greater opportunities for interactions with other hens and enrichment materials, which could affect their reaction to human approach. Conversely, the limited space and restricted capacity for retreat in CS due to the cage design might have led to shorter avoidance distances. Further research is necessary to explore the underlying factors contributing to these behavioral differences in hens and to develop strategies for accurately assessing their stress levels and comfort in the presence of humans across various housing environments.

The H:L ratio is commonly used to assess stress in laying hens, following the method by Gross et al. (1983). Earlier studies focusing on chickens suggest that a higher H:L ratio is related to higher levels of environmental stress (McFarlane and Curtis, 1989). Cotter (2015) found that H:L ratios observed in enriched cage systems (ECS) were significantly higher than those recorded in AS or CS, suggesting that hens in ECS may experience greater stress levels than those in either of the other systems (Cotter, 2015). However, another study recorded consistent H:L ratios in both conventional and ECS, suggesting that stress levels were low and comparable, likely due to the birds' prolonged familiarity with their cage mates and environment throughout the study's 11-month production period (Tactacan et al., 2009). Similarly, studies on laying hens have shown that stocking density does not affect hematological values, such as leukocyte differential counts and the H:L ratio (Thaxton et al., 2006; Tuerkyilmaz, 2008). The impact of feed restriction on immune parameters, including blood values, is also debated, with some studies finding effects (de Jong et al., 2002; Khajavi et al., 2003), while others do not (Hocking et al., 1994; Liew et al., 2003; Fassbinder-Orth and Karasov, 2006). These variations may be influenced by stress-related factors, such as the duration and intensity of stress exposure, and measurement-related factors, such as the specific indicators used and the timing of measurements (Mumma et al., 2006). In our study, the H:L ratio observed in the AS was greater than that in the CS, which may be due to the increased physical activity and movement inherent in AS. However, further research is needed to fully understand the specific factors within different housing systems that contribute to specific stress responses.

While the WBC count alone does not directly assess stress in laying hens, the granulocyte and agranulocyte components provide insight into the immune status of birds and their ability to respond to infections and other health challenges (Alabi et al., 2014). Thus, the WBC count is more effective at indicating a bird's capacity to combat diseases rather than assessing stress related to physical and physiological factors. The higher WBC count observed in AS hens may suggest a stronger immune response or greater exposure to pathogens, likely resulting from the more dynamic environment of the AS. Cotter (2015) found that the normalized total WBC frequency distribution for ECS significantly differed from those of AS and CS, with each group possessing a distinct distribution. This underscores the potential impact of housing conditions on immune responses. Moreover, the findings align with those of Thaxton et al. (2006), who also reported that housing environments significantly influence hematological parameters in laying hens. As immune function is crucial to enhancing laying hen welfare and health, these findings demonstrate the importance of further research into the impact of different housing environments on their immune status.

Evaluating blood characteristics and serum CORT levels can provide valuable insights into the physiological responses of laying hens to different housing environments (Maxwell, 1993). Mashaly et al. (1984) demonstrated that higher stocking densities increased plasma CORT levels in hens, whereas Davis et al. (2000) reported no change in CORT levels with increased stocking density. Additionally, Tactacan et al. (2009) reported that plasma CORT levels were equivalent in birds housed in CS and ECS, implying that stress levels were similar between the two caging systems. However, it is important to note that the levels of CORT in the blood can be influenced by various factors, including the time of day, bird handling, and individual variability, which may obscure subtle differences between the housing systems (Beuving and Vonder, 1978; Tactacan et al., 2009). Additionally, factors such as the method and timing of blood sampling, the hens' previous exposure to stressors, and even the ambient temperature at the time of collection can significantly impact CORT levels (Beuving and Vonder, 1978). Therefore, while our study did not find significant differences in CORT levels between CS and AS hens, the possibility that subtle variations were masked by external factors cannot be entirely excluded.

It is well-established that age and housing system are key factors influencing egg quality (Ahmadi and Rahimi, 2011; Holt et al., 2011). Housing systems affect egg weight by influencing feed efficiency and controlling the environment, while age impacts egg weight through improved nutrient utilization and physiological maturity, with older hens generally producing heavier eggs (Zita et al., 2009). This age-related increase in egg weight is supported by various studies (e.g., Bozkurt and Tekerli, 2009; Krawczyk, 2009). Regarding housing systems, Lewko and Gornowicz (2011) reported that eggs from CS are heavier than eggs from litter systems, and Philippe et al. (2020) reported that laying rate and egg weight were significantly lower in AS than CS, likely due to higher levels of animal activity and competition for resources in AS. These findings are consistent with our study, where eggs produced in CS were heavier than those produced in AS. This may be the result of better feed efficiency, reduced physical activity, and a more controlled environment, benefits of CS noted by Widowski et al. (2022). However, some studies have presented contrasting findings. For instance, Zita et al. (2018) found that eggs from litter systems were heavier than those from cages, while Vits et al. (2005) reported heavier egg weights from floor pens than from CS. In these cases, lower egg weights in CS may be related to higher egg production rates, as suggested by Michel and Huonnic (2003). In contrast, studies by Ahammed et al. (2014) and Ghanina et al. (2020) found no significant differences in average egg weights between AS and CS. The inconsistencies between these studies may be due to variations in experimental conditions, including hen breed, age, diet, and environmental management, emphasizing the need for further investigations into the impact of housing systems on egg quality in which these factors are controlled for.

CORT is the primary glucocorticoid found in hen plasma, and its concentrations in avian eggs increase when this hormone is experimentally elevated in the mother's bloodstream (Hayward and Wingfield, 2004; Love et al., 2005) and under stimulated stress conditions during egg production (Saino et al., 2005). In birds, yolk CORT preferentially accumulates from plasma during early follicular development under low-stress conditions, while stress elevates its accumulation in later stages of egg formation (Okuliarová et al., 2010). For instance, seven days after experimental treatment, increased plasma CORT in Japanese quail led to elevated CORT levels in their egg yolks (Hayward and Wingfield, 2004). Factors such as stress (Lee et al., 2022), age (Widowski et al., 2022), and health status (Dai et al., 2019) can all influence hormone deposition in yolks. Our results, which show higher CORT levels in the eggs of hens in CS compared to those in AS at 48 weeks of age, align with the results of previous studies (Engel et al., 2019; Son et al., 2022). These elevated CORT levels are likely due to the cumulative stress effects associated with the housing conditions over time (Goymann, 2005). We specifically analyzed yolk CORT at 48 weeks because this period likely allowed enough time for any cumulative effects of the housing environment to manifest in the yolk CORT levels. The absence of significant differences in yolk CORT at 28 and 38 weeks suggests that other factors, such as age and environmental conditions, may also influence hormone levels during the laying period (Widowski et al., 2022). Although this study found differences between housing systems in yolk CORT, the specific mechanisms behind these variations were not investigated. We propose that the observed differences in CORT levels may be attributed to increased stress, which was also reflected in physical indicators, such as feather quality, and body condition, and behavioral responses. However, further research is needed to confirm this hypothesis and to fully understand the underlying mechanisms.

Conclusion

This study compared and evaluated the welfare and productivity of laying hens housed in cage and aviary systems by examining various parameters, including physical condition, behavior, blood properties, egg quality, and CORT levels in serum and egg yolks. While the CS offered certain benefits to productivity, it appears to restrict the hen's ability to engage in natural behaviors, as evidenced by increased feather damage and the expression of negative behaviors. The findings emphasize the necessity of conducting such in-depth comparisons while controlling for environmental variables to accurately assess the impact of different housing systems on hen welfare. Considering these controlled conditions, our current findings suggest that the AS provides a more favorable environment for laying hen welfare than the CS. These insights offer valuable guidance for future housing designs and management practices aimed at enhancing both animal welfare and egg production. However, as this study was conducted on hens from a single farm, larger studies across multiple farms are needed to confirm these findings and ensure broader applicability.

Disclosures

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Jungwon Lee reports a relationship with Pulmuone Co., Ltd. that includes: employment. The authors declare that there are no conflicts of interests If there are other authors, they 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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Disclosures

The authors declare that there are no conflicts of interest.

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