The effect of different cage densities on selected stress and welfare indicators in brown and white laying hens

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Received April 16, 2025
Accepted July 8, 2025

Abstract

This study investigated the impact of different cage densities (750 cm^2/hen, 535 cm^2/hen, and 375 cm^2/hen) on stress and welfare indicators in brown (Hyline Brown, HB) and white (Isa Tinted, IT) laying hens. The research focused on evaluating feather, health, and body condition scores, along with the heterophil-to-lymphocyte (H/L) ratio, as indicators of stress and welfare. Our results revealed a significant effect of cage density on feather scores, with higher densities correlating with increased feather loss across all body regions ($P < 0.01$). Furthermore, elevated cage densities were associated with a higher incidence of injuries in the cloaca and foot regions, as well as poorer body condition scores ($P < 0.01$). Notably, the HB hybrid consistently exhibited superior welfare indicators compared to the IT hybrid, as evidenced by higher feather scores, and higher body condition scores. With the increase in cage density, an increase in the H/L ratio was observed, and accordingly, an increase in stress intensity was determined ($P < 0.01$). These findings underscore the complex relationship between cage density, genotype, stress, and welfare outcomes in laying hens, emphasizing the need for further research to elucidate these interactions and develop targeted strategies for improving laying hen welfare in commercial production systems.

Feather score, housing, poultry industry

Quality genetic material selection and poultry housing conditions are crucial for achieving commercial production targets (Nicol et al. 2013; Özentürk and Yıldız 2021; Sharma et al. 2022). Continuous advancements in the poultry industry has led to changes in animal breeding and genetic trends, making the use of new layer hybrids with high efficiency and long production life essential for sustainability in production (Arulnathan et al. 2024). The health, performance, and welfare of laying hens are influenced by their environmental conditions (Özentürk and Yıldız 2021; Sharma et al. 2022). Chickens that are genetically better adapted to their environment are more likely to exhibit positive welfare-related behaviours, such as reduced fear, improved social interactions, and increased ability to cope with environmental challenges (Christensen et al. 2019; Skånberg et al. 2023). Therefore, determining the extent to which each hybrid is affected by environmental conditions provides important insights for breeders in selecting the most suitable genotype (Janczak and Riber 2015; Ziemiańska et al. 2020; Underwood et al 2021).

Achieving the optimum balance in determining cage stocking density is crucial for both welfare conditions and economic considerations (Roy et al. 2020; Wan et al. 2023). Despite the negative impact of high stocking density of laying hens on production performance, producers are inclined to maximize the use of unit area and increase economic income by increasing the number of animals in the cage (Özentürk and Yıldız 2020; Underwood et al. 2021). Additionally, these systems offer advantages in terms of economic and environmental sustainability due to their low resource use and carbon footprint (Kheiralipour et al. 2024). However, the importance of providing more space

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for animals has become increasingly recognized in recent years due to the growing concern for animal welfare and consumer preferences (Christensen et al. 2019; He et al. 2022). Animals require adequate space to exhibit their natural behaviours, and being able to do so enhances their welfare (Bhanja and Bhadauria 2018; Hemsworth and Edwards 2020; Sözcü et al. 2021). Moreover, increasing space per bird may reduce stress by minimizing social stressors such as competition for resources like food and water. Enhanced welfare can prolong the productive lifespan of laying hens, thereby increasing their long-term productivity (Arulnathan et al. 2024). Therefore, research to determine the most suitable settlement density is crucial to ensure both economically ideal production and improved animal welfare. Such research can provide practical guidance to producers, enabling them to optimize cage placement density for the health and welfare of laying hens.

Scoring practices, which assess the presence of feathers and the injury status of laying hens, have become increasingly important recently as they are easy to apply and non-invasive (Saraiva et al. 2020; Özentürk et al. 2023; Weimer et al. 2019). Feather condition is a key indicator of welfare, and feather loss in animals is often attributed to environmental conditions (Weimer et al. 2019) as is also feather pulling and pecking behaviour (Campe et al. 2018; Baker et al. 2022). These behaviours can vary among individuals (Sözcü et al. 2021; Sokołowicz et al. 2023), indicating a genetic influence. The presence of feathers in caged chickens protects them from abrasions caused by cage equipment and reduces the risk of injury. Feather loss and damage can also lead to cannibalism, resulting in injuries and deaths (Schwarzer et al. 2022; Tok et al. 2022). Besides the economic losses from deaths, the increased feed consumption by animals to maintain body temperature due to feather loss can raise operational costs (Falker-Gieske et al. 2020). Additionally, stress from feather pecking can reduce egg production (Fijn et al. 2020). Furthermore, leukocyte components are reliable indicators of stress in poultry, with the heterophil-to-lymphocyte (H/L) ratio in peripheral blood being an important parameter used to determine stress (Gross and Siegel 1983; Lentfer et al. 2015).

This research aimed to assess the stress and welfare levels of brown and white laying hens raised in three different cage densities. The study aimed to determine the most suitable cage density for both genotypes based on the considered stress and welfare indicators.

Materials and Methods

The research was conducted at the Ataturk University Food and Livestock Application and Research Center, Poultry Unit and was ethically approved by the Ataturk University Animal Experiments Local Ethics Committee (Protocol number: 2022/5, Decision number: 71) on 27.04.2022.

Animals and management

White laying Isa Tinted (IT) and brown laying Hyline Brown (HB) hybrids which were 60 weeks old and reared under identical conditions in a floor-type poultry house, were used in the study. The experiment involved two different genotypes (IT and HB) and three cage density arrangements (5 hens/cage, 7 hens/cage, and 10 hens/cage). A total of 396 hens, 198 from each hybrid, were distributed in an equal number of cage compartment for each density group. Low housing density (CD5) was set as 750 cm^2/hen, normal cage density (CD7) as 535 cm^2/hen and high cage density (CD10) as 375 cm^2/hen. The allocation of animals to cages was randomized. A total of 120 animals, with 20 animals from each genotype × housing density subgroup, were evaluated to measure stress and welfare indicators.

Each conventional cage compartment had identical dimensions with a depth of 60 cm, width of 62.5 cm, rear height of 46 cm, front height of 51 cm, and feeder length of 62.5 cm. The floor was sloped at 7°. Ventilation was achieved through windows on the side walls, ventilation shafts on the ceiling, and a 140 cm × 140 cm electric negative pressure fan. Temperature was maintained between 16–24 °C using sensors connected to the ventilation and heating systems. Lighting consisted of fluorescent lamps providing white light for 16 h daily. During the production period from 46–65 weeks, hens were fed *ad libitum* with 2nd period egg feed (2720 ME 15.83 HP), all in granular form.

Determination of welfare indicators

In the study, feather, health scores, and body condition scores were used as welfare indicators (EFSA 2023; Özentürk et al. 2023; Tauson et al. 2005). At 60 weeks of age, a total of 120 hens, with 20 hens from each genotype and cage density group, were visually scored individually. Feather score was conducted using two

methods: evaluating the body as a whole and evaluating body parts separately. Feathering in six different parts of the body (neck, breast, back, wing, tail, and cloaca) was assessed both individually and as a total score. Scoring for feather condition ranged from 1 to 4, with scores of 2 and below indicating significant damage to the feathers, and scores of 10 to 12 in the total scoring indicating significant feather loss throughout the body (Table 1) (Tauson et al. 2005). Scores of 3 and above regionally, and 18 to 20 in the total scoring, indicate good feather condition. To assess health status, the comb and cloaca region, and the foot related to bumblefoot syndrome were evaluated. Scoring ranged from 1 to 3, with lower scores indicating severe injury, wear, aggressive behaviour in the flock, swollen foot syndrome, and poor body condition (Table 2) (Tauson et al. 2005; Grafl et al. 2017; Özentürk et al. 2023).

Table 1. Description of the scoring scheme used for the assessment of feathers.

Indicator/Score	Feather loss
1	> 75% of the feathers of the body region missing
2	> 50% and < 75% of the feathers of the body region missing
3	> 25% and < 50% of the feathers of the body region missing
4	No feather loss or < 25% of the feathers of the body region missing

Table 2. Description of the scoring scheme used for the assessment of integument condition.

Indicator/Score	Comb and cloaca damage	Foot pad dermatitis	Body condition
1	Single or multiple injuries of > 1.0 cm	Swollen (dorsally visible)	Prominent keel bone ridge with scarce overall breast muscle
2	Multiple injuries of < 0.5 cm or single injuries of > 0.5 cm and < 1.0 cm	Necrosis or proliferation of epithelium or chronic bumble foot with no or moderate swelling	Relatively well-developed breast muscle with a distinct protuberance at the keel bone
3	No injury, only single injury of < 0.5 cm diameter or length	Feet intact, no or minimal proliferation of epithelium	Well-developed relatively round breast muscle with limited protuberance at the keel bone

Determination of stress level

The heterophil-lymphocyte ratio (H/L) was used to assess stress levels in the study. At 60 weeks of age, blood samples were collected from under the wing vein (vena cutanea ulnaris) of 120 hens (20 randomly selected hens from each hybrid and cage density group), excluding those used for welfare indicator assessments. Blood smears were prepared, air-dried, and stained using the May-Grünwald-Giemsa method (Gross and Siegel 1983). A drop of cedar oil was applied to the top of the smear, where the blood was thinly spread. Leukocyte types were observed under a light microscope at × 100 magnification, and the different leukocyte types (heterophils, lymphocytes, monocytes, basophils, and eosinophils) were recorded by counting a total of 100 white blood cells from the smear edge and centre. The H/L ratio was calculated by dividing the number of heterophils by the number of lymphocytes (Özentürk and Yıldız 2021).

Statistical analysis

All statistical analyses were conducted using the SPSS software package (v.20.0). In the research, feather and health scores were assessed in different parts of the body using a Likert scale. Kruskal-Wallis H test, a non-parametric test, was employed to analyse the cage density (CD5, CD7, CD10). Mann-Whitney U test, another non-parametric test, was used for genotype (IT, HB) and pairwise comparison of cage densities. By performing Shapiro-Wilk normality test, it was determined that the data did not exhibit a normal distribution. Additionally, Chi-square (χ^2) test, a non-parametric test, was applied to compare genotype and frequency subgroups. The effect of hybrid and cage density on H/L ratio values were examined by GLM procedure and repeated measures analysis of variance was performed for H/L ratio data from blood cells in determining stress levels.

In statistical notation, the model was expressed as:

$Yijkl=\mu+ai+bj+(ab)ij+eijkl$

where:

- *Yijkl* is the value of any of the parameters,
- *ai* is the hybrid effect (IT and HB),
- *bj* is the effect of cage density (750 cm^2, 535 cm^2, and 375 cm^2),
- *(ab)ij* is the interaction of hybrid (i) and cage density (j), and
- *eijkl* represents the error due to chance with a mean of 0 and variance of σ^2_e ($N\sim[0, \sigma^2_e]$)

Table 3. The effects of cage density on feather and health scores in laying hen hybrids.

	IT	HB	CD5	CD7	CD10	*P* value				
						Genotype	Cage Density	CD5 CD10	CD5 CD10	CD7
	Feather score (mean ± standard error of the mean)									
Neck	2.78 ± 0.09	2.60 ± 0.07	3.03 ± 0.08^{a}	2.78 ± 0.10^{b}	2.28 ± 0.08^{c}	0.179	< 0.001	0.041	< 0.001	< 0.001
Breast	2.17 ± 0.10	2.07 ± 0.10	2.58 ± 0.09^{a}	2.08 ± 0.12^{b}	1.70 ± 0.11^{c}	0.545	< 0.001	0.001	< 0.001	0.029
Cloaca	1.97 ± 0.12^{y}	2.77 ± 0.15^{x}	3.08 ± 0.19^{a}	2.05 ± 0.15^{b}	1.98 ± 0.13^{b}	< 0.001	< 0.001	< 0.001	< 0.001	0.855
Back	2.02 ± 0.12^{y}	2.63 ± 0.11^{x}	2.95 ± 0.14^{a}	2.25 ± 0.13^{b}	1.78 ± 0.11^{c}	< 0.001	< 0.001	0.001	< 0.001	0.007
Wing	2.05 ± 0.10^{y}	2.60 ± 0.12^{x}	2.93 ± 0.12^{a}	2.48 ± 0.09^{b}	1.58 ± 0.11^{c}	0.001	< 0.001	0.004	< 0.001	< 0.001
Tail	1.52 ± 0.08^{y}	2.32 ± 0.14^{x}	2.70 ± 0.15^{a}	1.90 ± 0.12^{b}	1.15 ± 0.06^{c}	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Total	12.50 ± 0.37^{y}	14.98 ± 0.57^{x}	17.25 ± 0.50^{a}	13.53 ± 0.44^{b}	10.45 ± 0.36^{c}	0.002	< 0.001	< 0.001	< 0.001	< 0.001
			Health score							
Comb	2.38 ± 0.10	2.52 ± 0.08	2.58 ± 0.10	2.40 ± 0.12	2.38 ± 0.11	0.349	0.342	0.354	0.137	0.666
Cloaca	2.23 ± 0.11^{y}	2.78 ± 0.07^{x}	2.75 ± 0.09^{a}	2.60 ± 0.11^{a}	2.18 ± 0.13^{b}	< 0.001	0.001	0.285	< 0.001	0.010
Foot damage	2.38 ± 0.10	2.23 ± 0.08	2.73 ± 0.07^{a}	2.48 ± 0.10^{a}	1.73 ± 0.08^{b}	0.128	< 0.001	0.073	< 0.001	< 0.001
Body condition	1.80 ± 0.06^{y}	2.03 ± 0.08^{x}	2.28 ± 0.07^{a}	1.90 ± 0.06^{b}	1.58 ± 0.08^{c}	0.019	< 0.001	< 0.001	< 0.001	0,002

IT = Isa Tinted; HB= Hyline Brown; CD5 = Cage density - 5 hens/cage; CD7 = Cage density - 7 hens/cage; CD10 = Cage density - 10 hens/cage;

^{a,b,c} = Values with different superscripts within a row differ significantly (Cage density); ^{x,y}= Values with different superscripts within a column differ significantly (Genotype)

Results

The mean feather, health, and body condition scores of Isa Tinted (IT) and Hyline Brown (HB) hybrids are presented in Table 3. Significant differences in feather scores between genotypes were found in all regions except the neck and breast ($P < 0.01$). The total feather score was 12.50 ± 0.37 for IT and 14.98 ± 0.57 for HB, indicating that the white layer hybrid showed more feather loss overall ($P < 0.01$). The highest feather loss in white laying hens was observed in the tail region, while in brown laying hens, it was observed in the breast region. Health score analysis revealed higher injury rates in the cloaca region of the IT hybrid, with a significant difference between genotypes ($P < 0.01$). The body condition score was significantly higher in the brown layer HB hybrid ($P < 0.05$).

Table 3 also presents the mean feather, health, and body condition scores for different cage density groups. Significant differences were observed in feather scores between cage density groups in all body regions ($P < 0.01$). The total feather score was determined as 17.25 ± 0.50, 13.53 ± 0.44 and 10.45 ± 0.36 for CD5, CD7 and CD10, respectively. As cage density increased, feather loss increased significantly ($P < 0.01$) in all body parts except the cloaca. The score for CD5 group in the cloaca region was significantly higher than the other groups ($P < 0.01$), while the difference between CD7 and CD10 groups was found to be non-significant ($P > 0.05$). Health score analysis showed no significant difference

Table 4. Feather and health scores of laying hybrids in genotype x cage density groups.

	IT			HB		
	CD5	CD7	CD10	CD5	CD7	CD10	*P* value
			Feather score (mean ± standard error of the mean)				
Neck	3.10 ± 0.12^{a}	2.85 ± 0.17^{ab}	2.40 ± 0.13^{cd}	2.95 ± 0.09^{ab}	2.70 ± 0.11^{bc}	2.15 ± 0.08^{d}	< 0.001
Breast	2.40 ± 0.15^{ab}	2.05 ± 0.19^{b}	2.05 ± 0.17^{b}	2.75 ± 0.10^{a}	2.10 ± 0.16^{b}	1.35 ± 0.11^{e}	< 0.001
Cloaca	2.20 ± 0.26^{bc}	1.65 ± 0.17^{c}	2.05 ± 0.19^{bc}	3.95 ± 0.05^{a}	2.45 ± 0.21^{b}	1.90 ± 0.18^{bc}	< 0.001
Back	2.60 ± 0.20^{b}	1.90 ± 0.19^{cd}	1.55 ± 0.15^{d}	3.30 ± 0.16^{a}	2.60 ± 0.13^{b}	2.00 ± 0.15^{c}	< 0.001
Wing	2.55 ± 0.15^{bc}	2.25 ± 0.10^{c}	1.35 ± 0.11^{e}	3.30 ± 0.15^{a}	2.70 ± 0.13^{b}	1.80 ± 0.17^{d}	< 0.001
Tail	1.95 ± 0.14^{c}	1.40 ± 0.13^{d}	1.20 ± 0.09^{d}	3.45 ± 0.11^{a}	2.40 ± 0.13^{b}	1.10 ± 0.07^{d}	< 0.001
Total	14.80 ± 0.48^{b}	12.10 ± 0.57^{c}	10.60 ± 0.52^{d}	19.70 ± 0.42^{a}	14.95 ± 0.52^{b}	10.30 ± 0.52^{d}	< 0.001
			Health score (mean ± standard error of the mean)				*P* value
Comb	2.50 ± 0.17	2.35 ± 0.18	2.30 ± 0.15	2.65 ± 0.11	2.45 ± 0.17	2.45 ± 0.15	0.679
Cloaca	2.50 ± 0.17^{b}	2.40 ± 0.18^{b}	1.80 ± 0.16^{c}	3.00^{a}	2.80 ± 0.12^{ab}	2.55 ± 0.17^{b}	< 0.001
Foot damage	2.85 ± 0.08^{a}	2.65 ± 0.13^{ab}	1.65 ± 0.13^{c}	2.60 ± 0.11^{ab}	2.30 ± 0.15^{b}	1.80 ± 0.09^{c}	< 0.001
Body condition	2.05 ± 0.05^{b}	1.80 ± 0.09^{cd}	1.55 ± 0.11^{d}	2.50 ± 0.12^{a}	2.00 ± 0.07^{bc}	1.60 ± 0.11^{d}	< 0.001

IT = Isa Tinted; HB = Hyline Brown; CD5 = Cage density-5 hens/cage; CD7 = Cage density-7 hens/cage; CD10 = Cage density-10 hens/cage;
^{a,b,c,d,e} = Values within a row with different superscripts differ significantly at $P < 0.01$

in the comb region scores among all cage density groups ($P > 0.05$). However, more injuries were observed in the cloaca and foot regions in the high cage density (CD10) group compared to the other groups ($P < 0.01$). Body condition scores decreased as the presence of hens in the cages increased ($P < 0.01$).

Table 4 presents the scoring for genotype × cage density groups. Significant differences in feather scores were observed between groups in all body regions ($P < 0.01$). The highest total feather score was observed in the HB genotype under CD5 conditions, while the lowest was under CD10 conditions for both genotypes. Health scoring showed no significant difference in the comb region among all genotype × cage density groups

Table 5. Effect of genotype and cage density on H/L values.

Genotype	Cage density	H/L ratio (mean ± SE)
Isa Tinted	CD5	0.221 ± 0.011
	CD7	0.309 ± 0.011
	CD10	0.468 ± 0.011
	Total	0.333 ± 0.006
Hyline Brown	CD5	0.215 ± 0.011
	CD7	0.318 ± 0.011
	CD10	0.518 ± 0.011
	Total	0.350 ± 0.006
Total	CD5	0.218 ± 0.008^{a}
	CD7	0.314 ± 0.008^{b}
	CD10	0.493 ± 0.008^{c}
	Total	0.342 ± 0.005
		P value
Genotype		0.057
Cage density		< 0.001
Genotype x Cage density		0.040

SE = standard error of the mean; CD5 = Cage density-5 hens/cage; CD7= Cage density-7 hens/cage; CD10 = Cage density-10 hens/cage; H/L = Heterophil / Lymphocyte; ^{a,b,c}Different letters within one column are significantly different ($P < 0.001$)

($P > 0.05$). The lowest injury rate in the cloaca region was observed in the HB genotype under CD5 conditions, while the highest was in the IT genotype under CD10 conditions ($P < 0.01$). The foot damage score was lowest in CD10 conditions for both genotypes ($P < 0.01$). The highest body condition score was observed in the HB genotype under CD5 conditions ($P < 0.01$).

Table 5 presents the mean values of the H/L ratio for genotype and cage density groups. The effect of genotype on the H/L ratio was non-significant ($P > 0.05$). However, as cage density increased, the H/L ratio also increased ($P < 0.01$). The genotype × cage density interaction for the H/L ratio was also significant ($P < 0.05$).

Discussion

The total feather score values for the IT and HB hybrids were 12.50 ± 0.37 and 14.98 ± 0.57, respectively, with a significant difference. Tauson et al. (2005) defined a total body score of 10–12 and below as indicating serious feather loss. Following this criterion, our research determined that the IT hybrid exhibited serious feather loss (Tauson et al. 2005). This finding aligns with previous studies where differences in feather scores were observed between different layer hybrids (Onbaşılar et al. 2015). However, while the total feather score provides a general assessment of feather condition and welfare, it is essential to evaluate body parts separately to understand the underlying causes of feather loss (Campe et al. 2018; Özentürk et al. 2023). When assessing feather scores in different body regions, we found that the IT layer hybrid showed more feather loss in all regions except the neck and breast area. This aligns with previous studies reporting higher feather scores in brown laying hens in various body compartments (Onbaşılar et al. 2015; Campe et al. 2018). In the IT hybrid, the most significant feather loss was observed in the tail, cloaca, and back regions, which is consistent with findings of other studies (Hartcher et al. 2015; Saraiva et al. 2020). Feather pecking behaviour, a common cause of feather loss, is often targeted at the back, tail, and cloaca area, and it can be influenced by factors such as foraging behaviour and malnutrition (Baker et al. 2022). Feather loss in laying hens can be attributed to feather pulling and pecking behaviours, which can vary genetically (Nicol et al. 2013; Campe et al. 2018; Sözcü et al. 2021). Additionally, genes that determine feather pigmentation in chickens with different feather colours may also affect pecking behaviour (Bright 2007; Nicol et al. 2013). The white feather colour of the IT hybrid in our research compared to the brown feather color of the HB hybrid, may contribute to differences in feather scores, as reported in previous studies (Campe et al. 2018; Özentürk et al. 2023). Furthermore, white laying hens are often described as more active, panicky, and aggressive compared to brown laying hens, which could explain the observed inter-genotype differences in feather scores (Ziemiańska et al. 2020; Özentürk and Yıldız 2021). These differences in behaviour may lead to variations in feather pulling and pecking behaviours between genotypes.

The IT hybrid had lower health scores in the cloaca region compared to the HB hybrid, indicating more injuries in the IT hybrid. This finding aligns with the greater feather loss observed in the cloaca and tail regions of the IT hybrid. The smaller body size and cloacal structure of the IT hybrid may have contributed to an increased incidence of prolapse, especially in hens with high egg weights (Özentürk and Yıldız 2020). Moreover, the white feather colour in the IT hybrid could make any bleeding, such as that caused by prolapse or other injuries, more noticeable, potentially triggering pecking behaviour and leading to increased injuries in the cloaca area. Body condition scores were also lower in the IT hybrid compared to the HB hybrid. This difference may be attributed to the higher body weight of brown layers compared to white layers (Bahry et al. 2023). The higher body condition score in the HB hybrid is consistent with it having a higher welfare

level than the IT hybrid, as indicated by the feather and health scores considered in the study.

In the study, significant differences were observed in the total feather scores of laying hens across different cage density groups (CD5, CD7, and CD10). Feather loss increased as cage density increased. According to the criteria set by Tauson et al. (2005), serious feather loss was observed in laying hens raised under CD10 conditions. When assessing feather loss in different body regions, it was found that feather loss increased with increasing cage density in all regions. Laying hens in CD7 and CD10 conditions showed similar feather scores only in the cloaca region, while the CD5 group had a higher feather score, indicating better feather condition in lower density conditions. The study indicated that the effect of cage density on the comb region health score was non-significant, suggesting that cage density may not significantly impact this aspect of health. However, a higher incidence of injuries was observed in laying hens in the CD10 group compared to the CD5 and CD7 groups in terms of cloaca and foot scores, indicating that higher cage densities may increase the risk of injuries. Body condition scores decreased as cage density increased, with the CD10 group having the lowest body condition score. This finding is consistent with the idea that higher stocking densities can negatively impact the welfare of laying hens, affecting their body condition and overall health. The results of this study are in line with previous research, including that of Özentürk et al. (2023) and Weimer et al. (2019), which also found that feather loss and injuries increase with higher cage densities. Other studies have also highlighted the significant effect of stocking density on feather score (Khumput et al. 2018; Roy et al. 2020) and foot health (Fidan and Nazligül 2013), further supporting the importance of considering cage density in laying hen welfare management. Increased cage density can expose laying hens to social stressors like competition for resources such as food and water, leading to stress-induced behaviours. Stress can disrupt normal behaviours and physiological processes, increasing feather pecking and self-grooming behaviours which contribute to feather loss (Saraiva et al. 2020). Feather loss is also influenced by factors such as decreasing feeder distance per animal and increasing stress (Fidan and Nazlıgül 2013; Özentürk et al. 2023). Additionally, higher stocking densities can lead to increased competition in the cage, affecting social behaviour and increasing the tendency for aggressive pecking (Baker et al. 2022). Aggressive pecking behaviour often results in feather loss in the neck, head, and back areas of chickens (Grafl et al. 2017). Moreover, the corrosive effect of the cage, combined with increased competition during feeding, can cause feather loss and injuries, particularly in the chest area (Khumput et al. 2018; Özentürk et al. 2023).

In this study, the H/L values used to determine stress levels were 0.330 and 0.350 in the IT and HB hybrids, respectively, with no significant difference between the genotypes. According to (Nicol et al. 2013), H/L ratio values of 0.2, 0.5, and 0.8 indicate low, medium, and high stress levels, respectively (Gross and Siegel 1983). Based on this classification, both hybrids exhibited low to medium stress levels. In a study by Özentürk and Yıldız (2021), where three different genotypes were grown in two different cage densities, it was reported that two genotypes showed similar stress levels, whereas the third hybrid exhibited lower stress levels compared to the others. This is consistent with the literature, where differences between genotypes are often attributed to variations in live weights, behavioural needs, and environmental adaptability of laying hens (Özentürk and Yıldız 2021; Ziemiańska et al. 2020). However, contrary to these findings, our study did not observe significant differences in stress levels between genotypes. It is possible that environmental conditions in our study masked potential genetic effects on stress responses. Additionally, the methodology used to measure stress levels, including the timing of sample collection, may have influenced the results. In our study, measurements were taken at 60 weeks of age, while in most literature, measurements are typically taken at the

end of the laying period. Therefore, long-term effects of stress might have more clearly revealed genetic differences.

There was a significant increase in stress levels of laying hens as cage density increased. According to Clark et al. (2009) poultry blood typically has 26% heterophiles and 66% lymphocytes. According to criteria of Gross and Siegel (1983), we found that CD5 and CD7 cage densities induced low stress, while the CD10 group caused moderate stress in laying hens. Özentürk and Yıldız (2021) highlighted in their research that cage density affects stress levels, with stress increasing as cage density increases. This is supported by other literature sources that also report an increase in stress with higher stocking densities (El-Tarabany 2016; Hosseini et al. 2018). As cage density increases, the area per animal decreases, potentially limiting chickens' mobility and their ability to exhibit natural behaviours (Bhanja and Bhadauria 2018; Erensoy et al. 2021a; Sözcü et al. 2021). This limitation can lead to increased boredom and frustration (Nicol et al. 2013; Janczak and Riber 2015; Hemsworth and Edwards 2020). Additionally, higher cage density may reduce air quality and limit access to fresh air for the animals (Bilal et al. 2021; Erensoy et al. 2021b). Furthermore, increased animal density per unit area can lead to competition for feed and water resources (Baker et al. 2022). These factors collectively contribute to the increased stress levels observed in laying hens with higher cage densities. Increased stress levels can result in higher levels of corticosterone and an increase in the number of heterophils in circulation (Ziemiańska et al. 2020). It has been noted that stressed laying hens may experience a decrease in intracellular lymphocytes and IgA-secreting cells (Deng et al. 2012). This decrease in lymphocyte numbers may be due to glucocorticoid hormones' increased adhesion to circulating endothelial cells and lymphocytes (Dhabhar 2009).

In the study, we found a significant interaction between genotype and cage density for the H/L ratio. As cage density increased, the H/L ratio increased in both genotypes, with a higher increase observed in the CD10 cage density in the HB layer hybrid compared to the IT hybrid. Despite the same number of animals per cage area in both genotypes, the higher body weight of brown laying hens may have caused more stress in the HB hybrid as the body volume increased with cage density. This difference in the genotype × cage density interaction for the H/L ratio may be attributed to this factor.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

Thank you to Ataturk University Food and Livestock Application and Research Center for providing animal material and experimental area. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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