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Effects of Strain and Stocking Density on Performance, Egg Size Distribution, Egg Quality, and Welfare in Laying Hens Housed in Enriched Cage System

ABSTRACT

This study assessed the effect of strain and enriched cage stocking density on performance, egg size distribution, egg quality, and welfare status in laying hens. Lohmann brown (LB) and Lohmann LSL Classic (LW) strains at 20 weeks of age were allotted to different cage stocking densities, with 1016, 762, or 610 cm² of cage floor area per hen. Live body weight at the age at sexual maturity and at 52 weeks of age, feather condition, and some egg quality parameters differed between hen strains and among stocking densities ($p < 0.01$; $p < 0.05$). However, age at sexual maturity, livability, egg production, heterophil-to-lymphocyte ratio, and duration of tonic immobility were similar between hen strains and among stocking densities ($p > 0.05$). Furthermore, egg size distribution was similar between hen strains ($p > 0.05$), but dissimilar among stocking densities ($p < 0.01$). Rectal and comb temperatures differed between hen strains and among stocking densities, respectively ($p < 0.01$; $p < 0.05$). The age of hens influenced egg quality variables (except for egg weight and shape index), feather condition, and body region temperatures ($p < 0.01$). These results suggest that an enriched cage floor area of up to 610 cm² per hen does not compromise production performance and welfare status, except for the body weight of laying hens. Additionally, the two strains might be at similar levels regarding overall performance and welfare status, excluding body weight and feather condition.

INTRODUCTION

In many developed countries, the production systems for laying hens started to evolve in the 1920s, highly driven by the demand for the quantity and quality of eggs and ethical concerns about some systems (Tainika & Şekeroğlu, 2020). For the latter reason, it has been emphasized that every system has limitations in ensuring a friendly environment for the birds (Lay *et al.*, 2011). Enriched cages are currently under massive criticism, having already been banned in some countries. Notably, there have been debates since 2018 ("end cage age") to place a ban on enriched cages in the European Union (EU) (EFSA, 2023).

The developments in production systems for laying hens seem to be accompanied by the improvements in genetic selection breeding programs for the hens as well. Some authors have reported genetic disparities in some variables in laying hens, such as egg production (Ketta *et al.*, 2020; Rakonjac *et al.*, 2021; Sharma *et al.*, 2022), mortality (Rakonjac *et al.*, 2021), egg quality parameters (Krawczyk *et al.*, 2023), body temperature (Tainika *et al.*, 2024a), feather condition score (Tok *et al.*, 2022; Tainika *et al.*, 2024a), stress (Hassan *et al.*, 2023) and fear (Wei *et al.*, 2022) responses.

It has been fully established that the rearing environment can modify production and welfare outcomes across laying hen strains.



In the poultry industry, stocking density is one of the significant environmental and management factors regardless of the housing system. There are some reports indicating the influence of cage stocking density in laying hens. For instance, 552.3 cm² of cage floor area per hen reduced egg production, age at sexual maturity, and body weight at sexual maturity age compared to 736.3 and 1104.5 cm² (Erensoy *et al.*, 2021); 500 cm² per hen reduced body weight, egg weight, hen-day egg production, egg surface area, and yolk color as compared to 667, 1000, and 2000 cm² per hen (Saki *et al.*, 2012); and 257.1 cm² per hen decreased egg yield and egg weight, but increased shell-breaking strength compared to 360 cm² per hen (Jahanian & Mirfendereski, 2015). However, stocking density was found not to affect laying rate, egg weight, shape index, and shell thickness (Guo *et al.*, 2012), and body weight at 19 weeks of age, and egg quality parameters (Erensoy *et al.*, 2021), and hen day egg production (Weimer *et al.*, 2019).

Furthermore, it is well-established that the age of hens can influence egg quality traits (Yilmaz Dikmen *et al.*, 2017; Yurtseven *et al.*, 2021). Egg-laying time can also have a significant impact on egg weight (Tůmová *et al.*, 2009; Krawczyk *et al.*, 2023)

Regarding some reports on welfare measures, the feather condition of hens decreased as cage floor area per bird decreased from 1104.5 to 552.3 cm² (Erensoy *et al.*, 2021), 720 to 514.28 cm² (Tok *et al.*, 2022), and from 923 - 955 to 465 - 484 cm² (Weimer *et al.*, 2019). A high-stress response in hens was also found at 423 cm² per hen as compared to 564 cm² and 846 cm² per hen (Wan *et al.* 2023). However, the rectal temperature in hens did not differ between 398, 543, and 586 cm² per hen (Guo *et al.*, 2012). In addition, it is well-known that hen age can impact feather conditions in layer chickens (Weimer *et al.*, 2019; Tok *et al.*, 2022; Tainika *et al.*, 2024a).

Most of the previous studies regarding stocking density focused on conventional cages, leading to a scarcity of literature about furnished cages. Hartcher & Jones (2017) emphasized that even enriched cages do not allow birds to express all their behaviors. For this reason, it is important to determine "exactly how high the stocking density should be for the specific breeds" (Geng *et al.*, 2020), regarding the enriched cages to have a negative impact on hen performance and welfare. Thus, enriched cages should be fully studied as much as conventional cages. This will provide clear evidence on the effects of enriched cages in enhancing the performance and welfare of hens compared to conventional cages.

It has been hypothesized that laying hen strains vary in how they adapt to increasing stocking densities. This would be reflected in differences in performance, egg quality traits, and welfare measures between hen strains. It is also argued that increased cage floor area per bird (low stocking density) would enhance the production and welfare variables of hens, since the additional space would lead to enhanced behavioral expression of birds and physiological responses, which might be associated with the production, egg quality, and welfare variables of hens. Therefore, this study examined the influence of strain and different stocking densities on the performance, egg quality traits, and welfare of laying hens housed in enriched cage system.

MATERIALS AND METHODS

Ethical approval

This study was conducted under the guidelines for animal experiments of the Ministry of Food, Agriculture and Livestock, Türkiye. Approval was granted by the animal experiments local ethics committee of Niğde Ömer Halisdemir University Rectorate (approval number: 2022/19).

Experimental groups

This study was carried out at the commercial laying hen unit of Niğde Ömer Halisdemir University, Ayhan Şahenk Agricultural Application and Research Center. The total animal material for the study was 360 hens, 180 hens of each strain – Lohmann Brown (LB) and Lohmann LSL Classic (LW). Three different stocking densities (floor area per hen) were applied: 1016 cm² per hen (low: LSD), 762 cm² per hen (moderate: MSD), and 610 cm² per hen (high: HSD). This corresponded to respectively 15, 20, and 25 hens per enriched cage unit. There were three replicates for each stocking density treatment. The birds were reared from 20 to 52 weeks of age in 3-multitier enriched cages of 240 cm × 63.5 cm × 60 cm (length, width, and height).

The birds of a specific category were within similar live body weight ranges and had perfect feathering at the start of the study (20 weeks of age). The initial hen live weights were 1128.07 g and 1446.28 g for LW and LB strains, respectively; and 1312.87 g, 1275.18 g, and 1281.35 g for the LSD, MSD, and HSD replicates, respectively.

Likewise, the enriched cage system had 8 stainless drinking nipples shared between back-to-back cage units, a feeder trough in front of the cages, and a wire mesh floor. The furnished cage items included a dark blue curtained nesting area of 40 cm × 33.5



cm × 30 cm in length × width × height, respectively, two horizontal perches, each 180 cm long, with a nail shortener and a scratchpad.

The study was set up in a fully environmentally-controlled barn. Ventilation, temperature, humidity, and light were controlled by fully automatic systems. These systems were adjusted for the optimum living conditions of the animals. In case of any deviation from the optimum environmental conditions, an alarm and visual warning were received, and emergency intervention was carried out. The light/darkness cycle recommended in the Lohmann breeder management guide for alternative housing (Lohmann, 2021) was applied. The light sources were warm white LED bulbs, 24- watts. Additionally, the regulatory requirements and the standard vaccination recommendations for the region were followed.

Regarding feeding, from 20 to 22 weeks of age, birds were given pre-laying feed containing: 17% CP, 2.00% Ca, 0.55% P, and 2800 kcal/kg metabolizable energy. From 23 to 52 weeks of age, layer feed containing: 16.26% CP, 3.58% Ca, 0.44% P, and 2800 kcal/kg metabolizable energy. Feed and water were provided *ad libitum*.

Data collection

Performance variables

Hen live body weight (HLBW): was determined after obtaining the individual hen weights on a scale of 0.1 g weighing precision in each replicate at 20 weeks of age, the age at 50% of egg production, and at 52 weeks of age (end of the study). HLBW was later determined on pen basis.

From the day (d) of the first egg, eggs laid by hens were recorded every day per replicate cage unit. Individual egg weights were measured weekly until the end of the study.

Age at sexual maturity (ASM): was established as the days when 50% of eggs were collected from the treatment replicate cage unit. Then the egg yield data was used to determine hen house and hen day egg production (HHEP and HDEP, respectively) per treatment group as follows:

$$\text{HDEP} = \frac{\text{Number of eggs produced during the period}}{\text{Number of hen days in the period}} \times \text{days}$$

$$\text{HHEP} = \frac{\text{Number of eggs produced during the period}}{\text{Number of hens present at that period}}$$

Furthermore, a digital scale of 0.01 g weighing precision was used to take the weight of each egg

while considering egg-laying times: 8:30 a.m., 12:00., and 3.30 p.m. Subsequently, the average egg weights were calculated at the end of the study.

Moreover, daily mortalities per treatment replicate were recorded, and the data was utilized to determine the percentage of livability as follows:

Livability = (Number of hens at 20 weeks of age – number of hens remaining at 52 weeks of age) / (number of hens at 20 weeks of age) × 100.

The United States Department of Agriculture (USDA) egg size distribution

The weights of individual eggs per replicate cage unit were segregated into the USDA egg size categories (USDA, 2000): small (42.0-49 g; Sm), medium (49-56 g; M), large (56-65 g; L), extra-large (65-70 g; XL), and jumbo (≥ 70 g; XXL). The categorization of eggs in each USDA egg size group was expressed as a percentage of the total number of eggs obtained from each treatment replicate cage unit.

Egg quality traits

Egg quality traits were evaluated every four weeks between 22 and 52 weeks of hen age. 15% of the eggs laid were randomly selected from each replicate, and egg quality traits were analyzed after 24 hours of storage. A weighing scale of 0.01 g precision was used to obtain the egg weights (g). Afterwards, a digital caliper (0.01 mm) was used to obtain values for the egg length and width, which enabled the determination of shape index (SI, %) as indicated below:

$$\text{SI} = \frac{\text{Egg width}}{\text{Egg length}} \times 100$$

Then the Orka Food Technology egg forcer reader (Israel) was used to determine the shell-breaking strength in "Kg. force". Later, the egg was broken on a glass table, enabling the determination of albumen and yolk heights using a manual micrometer (0.01 mm). This was followed by the determination of albumen length, albumen width, and yolk diameter using a digital caliper (0.01 mm). Likewise, the color of the yolk was scored with a DSM yolk color fan and albumen pH was evaluated with a manual pH meter. Moreover, some of the data obtained above was used to calculate the egg surface area (ESA, cm²), albumen index (AI, %), yolk index (YI, %), and Haugh unit (HU) using the following formulas:

$\text{ESA} = 3.9782 \times \text{egg weight in grams}^{0.70}$, as reported by Carter (1975).



$$AI = \frac{\text{Albumen height}}{(\text{Albumen length} + \text{albumen width})/2} \times 100$$

$$YI = \frac{\text{Yolk height}}{\text{Yolk diameter}} \times 100$$

HU = 100 log (albumen height - 1.7 egg weight^{0.37} + 7.57), as stated by Haugh (1937).

Furthermore, the presence or absence of meat and blood spots in the albumen and yolk was examined by visual observation of the eggs broken on the grass table, and the ratio of eggs with or without the inclusions was determined accordingly.

Shell thickness (mm) was determined as the average of the three shell thickness values (mm) obtained from three different regions of the eggs (i.e. blunt, center, and pointed portions of the egg), which were measured on shells without the shell membrane, using a metrical manual micrometer (0.01mm).

Welfare traits

Feather condition score (FCS) was scored using a 4-point (1-4) scale (Tauson *et al.*, 1984), which focuses on the degree of feather loss in body regions of the birds (neck, breast, back, wings, tail, and cloaca area). 1: loss of all feathers, 2: loss of half of the feathers, 3: loss of a third of the feathers, and 4: perfect feathering. A score of 6 and 24 indicated poor and perfect feathering, respectively. Feather scoring was performed between 21 and 52 weeks of hen age, every after 4 weeks. In total, 54 birds, with 2, 3, and 4 birds from each replicate of LSD, MSD, and HSD treatments, respectively, were randomly selected to determine the feathering condition.

Body region temperatures (°C): the rectal temperature was determined by a digital thermometer (MEDIX KD-106, China) kept in the cloaca of hens up to the point of temperature rise stabilization. The breast region, comb, and footpad surface temperatures of the hens were determined by an infrared thermometer (LOYKA DARK II, China). The body region temperatures were measured each time the feather scores of hens were obtained.

Duration of tonic immobility (TI, seconds): assessed at 52 weeks of hen age in 54 animals in total, specifically 2, 3, and 4 birds from each replicate of LSD, MSD, and HSD treatments, respectively. The determination of TI duration followed the procedures of Jones and Faure (1981). In short, the bird was laid on its back in a short-sided open rectangular vessel, with the head hanging in space, and held by gentle pressure on the breast. The hand was gently removed after 15 seconds. TI was

achieved in birds that did not right or get up 10 seconds after being released, and the time it took the bird to return to the normal position was recorded as the duration of TI in seconds by a remote observer using a timer. If TI did not occur after 3 repeated attempts (TI inductions), the bird was assumed to be insusceptible and a score of 0 was given. The TI test period was limited to 10 minutes at most, and the TI duration was taken as 600 seconds for birds that did not return to the normal position at the end of this period (Jones & Faure, 1981). TI duration testing was carried out by an experienced researcher (Ph. D.) in the assessment of poultry welfare.

Blood collection and blood parameters (%): a 2-cc sterile syringe was used to collect blood samples from the wing vein of all birds tested for TI. Subsequently, a drop of blood was put on a smear slide and the blood drop was run by another slide to draw a thin blood smear. The smear blood slides were later taken to the laboratory. Subsequently, the staining process including May-Grunwald and Giemsa stains was conducted on the smear blood slides and the number of 100 blood leukocytes (lymphocytes, monocytes, heterophil, eosinophils, and basophils) were counted under a microscope. This was followed by determining the H/L ratios (Gross & Siegel, 1983), using the number of heterophils (H) and lymphocyte (L) cells for each bird.

Statistical analysis

The assumption of normality of the data was examined with the Kolmogorov-Smirnov test, and the homogeneity of variances was examined with the Levene test. Logarithmic transformation was performed on variables that did not meet the assumptions (blood parameters except eosinophil and body weight at 52 weeks of age). For the cases where the assumptions were met, variance analysis was applied to the data under the General Linear Model procedure. Duncan's multiple comparison test was used to determine within group differences. The effects examined in the research and the interactions of these effects are given in the model below:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + \alpha\beta_{ij} + \alpha\gamma_{ik} + \alpha\delta_{il} + \beta\gamma_{jk} + \beta\delta_{jl} + \gamma\delta_{kl} + \alpha\beta\gamma_{ijk} + \alpha\beta\delta_{ijl} + \alpha\gamma\delta_{ikl} + \beta\gamma\delta_{jkl} + \alpha\beta\gamma\delta_{ijkl} + \varepsilon_{ijklm}$$

In the model: μ : population mean, α_i : i. age effect, β_j : j. genotype effect, γ_k : k. stocking density effect, δ_l : l. egg-laying time effect (morning, noon, afternoon), $\alpha\beta_{ij} + \alpha\gamma_{ik} + \dots + \alpha\beta\gamma\delta_{ijkl}$: interaction effect, ε_{ijklm} : random error ($\varepsilon \sim N(\mu, \sigma^2)$)



The variables examined in the study were analyzed with 3 different models depending on whether they were affected by external factors: analyzes of live weight, age at sexual maturity, livability, lifespan, and egg production were done by Model 1; analyzes of egg quality traits, blood parameters, plumage score, and some temperature variables were done by Model 2; and finally analyzes of egg weight and egg laying percentage characteristics were done by Model 3.

In Model 1, the effect of genotype (β_j), the effect of stocking density (γ_k), and the interaction of these effects ($\beta\gamma_{jk}$) were investigated by analysis of variance. In Model 2, α_i : i. effect of age, β_j : j. effect of strain, γ_k : k. the effect of stocking density and the interaction effects of these effects were investigated; and in Model 3, α_i : i. effect of age, β_j : j. effect of genotype, γ_k : k. Effect of stocking density, δ_l : l. effect of time, and the interactions of these effects were examined.

Whether there was blood or meat in the egg yolk and egg white, and whether the egg quality groups

were dependent on the environmental conditions examined was examined by chi-square analysis. SPSS 29 statistical package program was used for descriptive statistical values (mean, std error, frequency, percentage), analysis of variance, Duncan's multiple comparison test, and chi-square analysis.

RESULTS

The data for performance traits of hens are presented in Table 1. The effect of strain on HLBW at sexual maturity age and 52 weeks of age was significant ($p<0.01$), with LB hens being heavier than LW hens. Nevertheless, ASM, livability, lifespan, HDEP, and HHEP did not differ between laying hen strains ($p>0.05$). Stocking density significantly affected HLBW at sexual maturity age and 52 weeks of age ($p<0.05$), HLBW was higher for LSD hens compared to MSD and HSD, which had similar values. However, stocking density did not affect ASM, livability, lifespan, HDEP, or HHEP ($p>0.05$).

Table 1 – Impact of strain and stocking density on live body weight, age at sexual maturity, livability, lifespan, and egg production in laying hens housed in enriched cages.

Factor		HLBW at ASM, g	HLBW at 52 weeks, g	ASM, d	Livability, %	Lifespan, d	HDEP, number of eggs	HHEP, number of eggs
Hen strain (HS)	LW	1493.55	1595.24	162.11	98.22	197.00	187.05	183.87
	LB	1856.39	1886.76	163.56	93.48	214.67	190.18	189.42
	p value	<0.001	<0.001	0.532	0.174	0.233	0.527	0.372
Stocking density (SD)	LSD	1701.50 ^a	1788.08 ^a	160.67	95.56	215.70	191.44	189.83
	MSD	1664.33 ^b	1729.53 ^b	162.00	96.67	206.60	191.98	190.25
	HSD	1668.70 ^b	1729.13 ^b	165.83	95.33	191.08	182.42	179.85
	p value	<0.047	<0.048	0.192	0.939	0.418	0.228	0.311
SEM		11.47	12.15	1.10	1.59	9.10	2.26	2.92
HS×SD		0.423	0.339	0.998	0.434	0.811	0.695	0.688

Abbreviations: HLBW; hen live body weight, d: days, ASM: age at sexual maturity, LW; Lohmann LSL Classic, LB; Lohmann brown; SEM; standard error of mean, HDEP: hen day egg production, HHEP: hen house egg production, LSD: low stocking density, MSD: moderate stocking density, HSD: high stocking density, ×: interactions between different factors.

Means within the column with different letter superscripts differ significantly ($p<0.05$).

The average egg weight and egg-laying percentage results are shown in Table 2. The average egg weight did not vary between hen strains ($p>0.05$). In contrast, there was a significant decrease in average egg weight with the increase in stocking density ($p<0.01$). In addition, the average egg weight significantly increased with the increase in hen age ($p<0.01$). Also, the average egg weight was significantly the highest and the lowest at the 12:00 and 3:30 p.m. egg-laying times, respectively ($p<0.01$). There was no effect of stocking density, hen strain, and hen age on egg-laying percentage ($p>0.05$). However, the influence of egg-laying time on egg-laying percentage was significant ($p<0.01$), as it was highest and lowest at 12:00 and 3:30 p.m., respectively.

There were significant age × hen strain, age × stocking density, age × egg-laying time, age × hen strain × stocking density, and age × hen strain × egg-laying time interaction effects for average weight ($p<0.01$). Furthermore, there were significant age × egg-laying time, and hen strain × egg-laying time interaction effects for egg-laying percentage ($p<0.01$; Table 3).

The USDA egg size distribution results are shown in Table 3. Overall, most of the eggs laid were in the L category. Moreover, except for Sm eggs, M, L, XL, and XXL eggs were higher for LB than LW hens ($p<0.01$). There was a significant decrease in L, XL, and XXL and a significant increase in M eggs with the increase in stocking density ($p<0.01$). In addition,



Table 2 – Impact of age, strain, stocking density, and egg-laying time on average egg weight and egg-laying percentage in laying hens housed in enriched cages.

Factor		Average egg weight, g	Egg-laying percentage, %
Age (A, week)	20-31	57.99 ^c	32.97
	32-41	63.42 ^b	33.33
	42-52	64.19 ^a	33.33
	<i>p</i> value	<0.001	0.943
Hen strain (HS)	LW	62.03	33.33
	LB	62.16	33.09
	<i>p</i> value	0.073	0.808
Stocking density (SD)	LSD	62.65 ^a	33.15
	MSD	62.17 ^b	33.15
	HSD	61.70 ^c	33.33
	<i>p</i> value	<0.001	0.985
Egg-laying time (LT)	9:00 a.m.	62.05 ^b	38.67 ^b
	12:00 p.m.	62.54 ^a	51.19 ^a
	3:30 p.m.	59.94 ^c	9.78 ^c
	<i>p</i> value	<0.001	<0.001
	SEM	0.06	1.69
	A×HS	<0.001	0.943
	A×SD	<0.001	1.000
	A×LT	<0.001	<0.001
	HS×SD	0.098	0.985
	HS×LT	0.282	<0.001
	SD×LT	0.066	0.014
	A×HS×SD	<0.003	1.000
	A×HS×LT	<0.001	0.182
	A×SD×LT	0.126	<0.021
	HS×SD×LT	0.087	0.430
	A×HS×SD×LT	0.561	0.340

Abbreviations: LW; Lohmann LSL Classic, LB; Lohmann brown; SEM; standard error of mean, LSD: low stocking density, MSD: moderate stocking density, HSD: high stocking density, ×: interactions between different factors.

Means within the column with different letter superscripts differ significantly ($p < 0.05$).

while Sm, M, and L eggs significantly decreased with the aging of hens, the opposite trend was observed for XL and XXL. Furthermore, there was a significant impact of egg-laying time on egg size distribution ($p < 0.01$), with the highest number of L and XL eggs laid at 12:00, Sm and XXL eggs at 9:00 a.m., and M eggs at 3:30 p.m.

The egg quality traits results are presented in Table 4. There was a significant difference between hen strains regarding some egg quality traits. Shape index, shell-breaking strength, and shell thickness were greater in LB eggs, and albumen index, Haugh unit, and albumen pH were higher in LW eggs ($p < 0.01$). Egg weight, yolk index, and yolk color score did not differ between hen strains ($p > 0.05$). Also, there was a significant variation among stocking densities regarding some egg quality parameters ($p < 0.01$; $p < 0.05$). Egg weight was higher in LSD eggs, with similar values for MSD and HSD eggs; shape index was lowest in LSD eggs and highest in MSD eggs; and yolk color score

was lowest in LSD eggs and highest in HSD eggs. There was no influence of stocking density on shell-breaking strength, shell thickness, albumen index, Haugh unit, yolk index, and albumen pH ($p > 0.05$). Furthermore, age-associated changes were observed in egg quality traits ($p < 0.01$), except for egg weight and shape index ($p > 0.05$). Additionally, there was a significant interaction effect of age × hen strain on shell thickness, yolk index, and yolk color score ($p < 0.01$; $p < 0.05$), age × stocking density on egg weight and shell thickness ($p < 0.05$), hen strain × stocking density on shell-breaking strength, and albumen index ($p < 0.05$), and age × hen strain × stocking density on egg weight, shape index, and yolk color score ($p < 0.05$).

The meat-blood inclusions in albumen results are shown in Table 5. There was a significant difference between hen strains concerning meat and blood spots in the albumen ($p < 0.01$), with these spots only identified in LB eggs. Also, the percentage of eggs with meat and blood inclusions in albumen differed



Table 3 – Impact of strain, stocking density, age, and egg-laying time on USDA egg size distribution expressed as the number of eggs and ratio of eggs (%) in laying hens housed in enriched cages.

Factor		USDA egg size distribution					Total
		Sm	M	L	XL	XXL	
Hen strain	Overall, n	146 (1.6)	974 (10.9)	4767 (53.6)	2096 (23.6)	915 (10.3)	8898 (100)
	LB	65 (1.5)	477 (10.7)	2429 (54.3)	1032 (23.1)	469 (10.5)	4472 (100)
	LW	81 (1.8)	497 (11.2)	2338 (52.8)	1064 (24)	446 (10.1)	4426 (100)
	χ^2	4.730					
	p value	0.316					
Stocking density	LSD	35 (1.6)	192 (8.7)	1122 (51.1)	597 (27.2)	249 (11.3)	2195 (100)
	MSD	53 (1.7)	358 (11.6)	1604 (52.1)	713 (23.2)	351 (11.4)	3079 (100)
	HSD	58 (1.6)	424 (11.7)	2041 (56.3)	786 (21.7)	315 (8.7)	3624 (100)
	χ^2	54.888					
	p value	<0.001					
Age (Week)	20-31	135 (5.1)	679 (25.8)	1521 (57.7)	228 (8.7)	72 (2.7)	2635 (100)
	32-41	5 (0.2)	174 (5.9)	1577 (53.4)	824 (27.9)	371 (12.6)	2951 (100)
	42-52	6 (0.2)	121 (3.7)	1669 (50.4)	1044 (31.5)	472 (14.3)	3312 (100)
	χ^2	1623.373					
	p value	<0.001					
Egg-laying Time	9:00	92 (2.9)	371 (11.7)	1593 (50.2)	738 (23.3)	379 (11.9)	3173 (100)
	12:00	36 (0.8)	438 (9.1)	2606 (54.3)	1220 (25.4)	499 (10.4)	4799 (100)
	3:30	18 (1.9)	165 (17.8)	568 (61.3)	138 (14.9)	37 (4)	926 (100)
	χ^2	208.879					
	p value	<0.001					

Abbreviations: LW: Lohmann LSL Classic, LB: Lohmann brown, LSD: low stocking density, MSD: moderate stocking density, HSD: high stocking density, Sm: small (42.0-49 g), M: medium (49-56 g), L: large (56-65 g), XL: extra-large (65-70 g), XXL: jumbo (≥ 70 g), Significant difference at $p < 0.05$. The values in front of the brackets are the absolute number of eggs, and the values within the brackets are the percentages.

Table 4 – Impact of age, strain and stocking density on egg quality traits in laying hens housed in enriched cages.

Factor		Egg weight, g	Shape index, %	Shell breaking strength, Kg. f	Shell thickness, mm	Albumen index, %	Haugh unit	Yolk index, %	Yolk color score, DSM	Albumen pH
Age (A, week)	33	62.17	77.90	4.76 ^b	0.394 ^{bc}	10.17 ^{ab}	88.74 ^{ab}	40.15 ^c	12.33 ^a	8.71 ^b
	37	63.04	77.00	5.35 ^a	0.406 ^a	9.62 ^b	87.16 ^b	38.31 ^d	10.96 ^c	8.72 ^b
	41	63.60	77.10	5.03 ^b	0.387 ^c	10.67 ^a	90.13 ^a	43.77 ^{ab}	9.98 ^d	8.76 ^b
	45	63.19	77.31	5.06 ^b	0.405 ^a	9.94 ^b	86.87 ^b	43.40 ^b	12.09 ^a	8.86 ^a
	50	64.53	76.83	4.81 ^b	0.400 ^{ab}	10.80 ^a	90.55 ^a	44.68 ^a	11.46 ^b	8.90 ^a
	p value	0.159	0.165	<0.007	<0.001	<0.001	<0.007	<0.001	<0.001	<0.001
Hen strain (HS)	LW	63.25	75.90	4.89	0.394	10.70	90.32	41.74	11.32	8.82
	LB	63.36	78.56	5.12	0.403	9.77	87.07	42.38	11.41	8.75
	p value	0.882	<0.001	<0.009	<0.008	<0.001	<0.001	0.259	0.372	<0.002
Stocking density (SD)	LSD	64.60 ^a	76.64 ^b	5.05	0.401	10.14	88.42	42.14	11.17 ^b	8.83
	MSD	63.40 ^b	77.54 ^a	5.03	0.399	10.21	88.55	42.14	11.32 ^{ab}	8.79
	HSD	62.59 ^b	77.51 ^{ab}	4.94	0.397	10.31	88.94	41.97	11.50 ^a	8.77
	p value	<0.003	<0.031	0.505	0.457	0.838	0.861	0.906	<0.007	0.083
	SEM	0.24	0.15	0.05	0.002	0.12	0.43	0.24	0.07	0.01
	A×HS	0.402	0.980	0.963	<0.047	0.397	0.441	<0.007	<0.001	0.996
	A×SD	<0.029	0.205	0.244	<0.036	0.461	0.505	0.763	0.453	0.481
	HS×SD	0.951	0.626	<0.019	0.775	<0.050	0.064	0.245	0.102	0.965
	A×HS×SD	<0.047	<0.038	0.198	0.595	0.322	0.286	0.470	<0.011	0.911

Abbreviations: LW: Lohmann LSL Classic, LB: Lohmann brown; SEM: standard error of mean, LSD: low stocking density, MSD: moderate stocking density, HSD: high stocking density, ×: interactions between different factors. Means within the column with different letter superscripts differ significantly ($p < 0.05$).

among stocking densities ($p < 0.05$). The number of eggs without inclusions was lowest in LSD hens, which also had a higher number of eggs with meat spots in the albumen. MSD hens had the highest number

of normal eggs and also had the highest number of eggs with blood spots in the albumen. However, there was no age effect on meat and blood inclusions in the albumen ($p > 0.05$).



Table 5 – Impact of age of hens, laying hen strain, and stocking density on meat and blood inclusions in the albumen.

Factor		% of eggs with or without inclusions in the albumen				Total	χ^2	P
		Normal	Meat spotted eggs	Blood spotted eggs	Meat-blood spotted eggs			
Age, week	33	51 (94.4)	1 (1.9)	2 (3.7)	0 (0)	54 (100)	12.046	0.442
	37	47 (87)	4 (7.4)	2 (3.7)	1 (1.9)	54 (100)		
	41	50 (92.6)	4 (7.4)	0 (0)	0 (0)	54 (100)		
	45	48 (88.9)	5 (9.3)	0 (0)	1 (1.9)	54 (100)		
	50	49 (90.7)	5 (9.3)	0 (0)	0 (0)	54 (100)		
Hen strain	LB	110 (81.5)	19 (14.1)	4 (3)	2 (1.5)	135 (100)	27.551	<0.001
	LW	135 (100)	0 (0)	0 (0)	0 (0)	135 (100)		
Stocking density	LSD	51 (85)	9 (15)	0 (0)	0 (0)	60 (100)	13.516	<0.036
	MSD	84 (93.3)	3 (3.3)	3 (3.3)	0 (0)	90 (100)		
	HSD	110 (91.7)	7 (5.8)	1 (0.8)	2 (1.7)	120 (100)		
Total		245 (90.7)	19 (7.0)	4 (1.5)	2 (0.7)	270 (100)		

Abbreviations: LW; Lohmann LSL Classic, LB; Lohmann brown, LSD: low stocking density, MSD: moderate stocking density, HSD: high stocking density. The values in front of the brackets are the absolute number of eggs, and the values within the brackets are the percentages

The results for meat-blood inclusions in the yolk are indicated in Table 6. There was a significant difference between hen strains concerning meat and blood spots in the yolk ($p<0.01$), with these spots only being observed in LB eggs. However, there was no stocking density and age effect on meat and blood inclusions in the yolk ($p>0.05$).

The results for the FCS and body region temperatures are shown in Table 7. The feather condition was significantly poorer in LB compared with LW hens ($p<0.05$). In addition, LSD hens had significantly lower FCS ($p<0.01$) than MSD and HSD hens, whose scores were similar. Furthermore, FCS was reduced with the aging of hens ($p<0.01$). There was a significant interaction effect of age $\times$ hen strain and hen strain $\times$ stocking density on FCS ($p<0.01$).

There was a significant variation between strains regarding rectal temperature ($p<0.01$), which was

higher in LB than in LW hens. However, there was no significant difference regarding comb, breast region, and footpad surface temperatures between hen strains ($p>0.05$). There was a significant difference concerning comb temperature ($p<0.01$), which was lowest in HSD hens and highest in MSD hens. On the other hand, breast region, footpad surface, and rectal temperatures did not vary among the stocking densities ($p>0.05$). Meanwhile, age-related changes were observed for all the body region temperatures ($p<0.01$). Also, there was a significant interaction effect of age $\times$ hen strain, and age $\times$ hen strain $\times$ stocking density for comb temperature ($p<0.01$; $p<0.05$); hen strain $\times$ stocking density, and age $\times$ hen strain $\times$ stocking density on breast region temperature ($p<0.01$; $p<0.05$); and age $\times$ hen strain, age $\times$ stocking density, hen strain $\times$ stocking density, and age $\times$ hen strain $\times$ stocking density on footpad surface and rectal temperatures ($p<0.01$; $p<0.05$).

Table 6 – Impact of the age of hens, laying hen strain, and stocking density on meat and blood inclusions in the yolk.

Factor		% of eggs with or without inclusions in the yolk			Total	χ^2	P
		Normal eggs	Meat spotted eggs	Blood spotted eggs			
Age, week	33	44 (81.5)	0 (0)	10 (18.5)	54 (100)	12.835	0.118
	37	52 (96.3)	0 (0)	2 (3.7)	54 (100)		
	41	48 (88.9)	1 (1.9)	5 (9.3)	54 (100)		
	45	48 (88.9)	1 (1.9)	5 (9.3)	54 (100)		
	50	52 (96.3)	0 (0)	2 (3.7)	54 (100)		
Hen strain	LB	109 (80.7)	2 (1.5)	24 (17.8)	135 (100)	28.770	<0.001
	LW	135 (100)	0 (0)	0 (0)	135 (100)		
Stocking density	LSD	54 (90)	1 (1.7)	5 (8.3)	60 (100)	3.838	0.428
	MSD	85 (94.4)	0 (0)	5 (5.6)	90 (100)		
	HSD	105 (87.5)	1 (0.8)	14 (11.7)	120 (100)		
Total		272 (85)	43 (13.4)	5 (1.6)	320 (100)		

Abbreviations: LW; Lohmann LSL Classic, LB; Lohmann brown, LSD: low stocking density, MSD: moderate stocking density, HSD: high stocking density. The values in front of the brackets are the absolute number of eggs, and the values within the brackets are the percentages.



Table 7 – Impact of age, strain, and stocking density on feather score and body region temperatures (°C) in laying hens housed in enriched cages.

Factor		Feather score	Comb temperature	Breast region surface temperature	Footpad surface temperature	Rectal temperature
Age (A, week)	21	24.00 ^a	-	-	-	-
	25	24.00 ^a	-	-	-	-
	29	24.00 ^a	-	-	-	-
	33	24.00 ^a	36.71 ^a	36.70 ^c	36.20 ^a	41.16 ^a
	37	23.31 ^a	36.47 ^a	36.46 ^c	35.95 ^a	40.99 ^{ab}
	41	21.00 ^b	33.36 ^b	36.57 ^c	35.38 ^a	38.03 ^d
	45	20.67 ^b	32.94 ^{bc}	36.66 ^c	34.41 ^b	40.70 ^{bc}
	50	19.44 ^c	32.31 ^c	39.99 ^a	32.67 ^c	40.41 ^c
	52	19.61 ^c	26.81 ^d	39.28 ^b	27.51 ^d	41.17 ^a
	<i>p</i> value	<0.001	<0.001	<0.001	<0.001	<0.001
Hen strain (HS)	LW	22.33	32.23	37.70	33.95	40.08
	LB	22.12	32.98	37.52	33.42	40.74
	<i>p</i> value	<0.040	0.183	0.918	0.299	<0.001
Stocking density (SD)	LSD	21.70 ^b	33.08 ^{ab}	37.40	33.74	40.36
	MSD	22.48 ^a	33.53 ^a	37.94	33.66	40.39
	HSD	22.30 ^a	32.79 ^b	37.47	33.68	40.45
	<i>p</i> value	<0.004	<0.033	0.052	0.976	0.683
	SEM	0.12	0.22	0.13	0.23	0.10
A×HS		<0.002	<0.017	0.517	<0.001	<0.001
A×SD		0.139	0.805	0.885	<0.023	<0.008
HS×SD		<0.004	0.093	<0.020	<0.020	<0.001
A×HS×SD		0.333	<0.002	<0.002	<0.004	<0.001

Abbreviations: LW; Lohmann LSL Classic, LB; Lohmann brown; SEM; standard error of mean, LSD: low stocking density, MSD: moderate stocking density, HSD: high stocking density, ×: interactions between different factors.

Means within the column with different letter superscripts differ significantly ($p < 0.05$).

The results for the blood parameters and TI duration are shown in Table 8. There was no significant difference between hen strains and among stocking densities and their interaction concerning H/L ratio and TI duration ($p > 0.05$). However, monocyte and heterophil percentages differed between hen strains ($p < 0.01$; $p < 0.05$), and basophil percentages varied among stocking densities ($p < 0.05$).

DISCUSSION

The performance of hybrids or strains is inherited from the breeds (parents) from which they were developed. Therefore, inter-strain variability in production variables can be expected. Similarly, in the current study, HLBW varied between the strains, which is in line with some other reports (Sözcü et

Table 8 – Impact of strain and stocking density on blood parameters (%) and duration of tonic immobility (seconds) in laying hens housed in enriched cages.

Factor		Lymphocyte	Monocyte	Heterophil	Eosinophil	Basophil	H/L ratio	TI duration
Hen strain (HS)	LW	48.00	18.44	29.19	3.56	0.82	0.80	302.70
	LB	45.15	10.37	41.11	3.41	0.96	1.03	239.15
	<i>p</i> value	0.539	<0.002	<0.014	0.921	0.569	0.102	0.213
Stocking density (SD)	LSD	46.83	15.58	33.58	2.75	1.25 ^{ab}	0.90	267.92
	MSD	43.06	14.17	37.11	4.28	1.39 ^a	1.14	239.22
	HSD	49.08	14.00	33.33	3.25	0.33 ^b	0.75	296.21
	<i>p</i> value	0.191	0.568	0.836	0.262	<0.019	0.416	0.619
SEM		1.75	1.06	2.27	0.36	0.18	0.09	25.20
HS×SD		0.439	0.542	0.875	0.469	0.190	0.672	0.391

Abbreviations: LW; Lohmann LSL Classic, LB; Lohmann brown; SEM; standard error of mean, LSD: low stocking density, MSD: moderate stocking density, HSD: high stocking density, H/L ratio: heterophil-to-lymphocyte ratio, ×: interactions between different factors.

Means within the column with different letter superscripts differ significantly ($p < 0.05$).



al., 2021; Tainika *et al.*, 2024b). However, the values for each strain were not in the range of the industry targets, as reported in the Lohmann management guide (Lohmann, 2021). For instance, in the guide, at 52 weeks of age, the HLBW range is stated to be between 1916 - 2034 g and 1685 - 1825 g for LB and LW, respectively. ASM was not different between the strains and was slightly higher than the industry targets for both strains (150 – 160 days) (Lohmann, 2021). The strains also did not vary regarding livability, which is similar to the results reported by some authors (Rakonjac *et al.*, 2021; Tainika *et al.*, 2024b). Still, the individual values were better than the industry targets (Lohmann, 2021) during the laying period (90 - 92%).

Furthermore, the strains had similar egg yields, which is in line with Rakonjac *et al.* (2021), but slightly contradicts Tainika *et al.* (2024b), who reported the disparity in hen day egg production between hen strains. The present study, however, the strain values were lower than the industry targets. For example, hen house egg production is reported as 199 and 201.5 eggs at 52 weeks of age for LB and LW (Lohmann, 2021), respectively. Overall, factors including differences in the study region, management level, housing conditions and environment, and feeding could be the reasons for the discrepancy in the results between the present study, the industry targets, and some other research studies.

Stocking density is one of the significant environmental stressors in the poultry industry. Several authors have reported a decrease in HLW as the stocking density increased (Bozkurt *et al.*, 2006; Jensen, 2019; Erensoy *et al.*, 2021; Zaazaa *et al.*, 2023), which is in line with the current study. Contrary to these studies, Anderson & Adams (1992) found no differences in body weight in hens reared at 221, 249, 277, and 304 cm² per hen. Also, von Eugen *et al.* (2019) reported no body weight differences at low (500 - 1000 - 1429 cm² per bird), moderate (167 - 333 - 500 cm² per bird), and high (56 - 111 - 167 cm²) densities.

The current study did not find variations in stocking densities regarding ASM, as reported by Erensoy *et al.* (2021). Also, the present study did not identify differences in stocking densities concerning egg production, which is consistent with some other studies (Şahin *et al.*, 2007; Zaazaa *et al.*, 2023). In contrast, some reports have indicated the effect of cage stocking density on egg production, whereby egg production was depressed with the increased stocking density (Altan *et al.*, 2002; Sarica *et al.*, 2008; Kang *et al.*, 2016; Erensoy *et al.*, 2021).

These variations in results of stocking density on production variables are linked to the specific cage floor area per hens utilized in the specific studies. Notably, the effect of increased stocking density has been associated with competition for cage floor area usage to perform some activities (Widowski *et al.*, 2016). This can frustrate the hens and consequently influence their physiological responses, adversely affecting production variables. Some studies determined no effect of stocking density on livability (Anderson & Adams, 1992; Carey *et al.*, 1995), which aligns with the present study. However, Altan *et al.* (2002) and Sarica *et al.* (2008) reported increased mortality as stocking density increased. In particular, Sarica *et al.* (2008) emphasized that the higher mortalities were associated with increased pecking behavior in cages with 667 and 500 cm² per hen as compared to those with 1000 and 2000 cm² per hen. Wilson *et al.* (1967) linked mortalities due to cage stocking densities to cannibalism. Furthermore, it is argued that contradictory findings regarding the effect of stocking density might be related to the differences in the cage floor area per bird and strains used in the specific studies, among other factors.

In the current study, the average egg weight had no genetic basis, which contrasts with some other reports (Lordelo *et al.*, 2020; Hammershøj *et al.*, 2021; Krawczyk *et al.*, 2023; Tainika *et al.*, 2024b). These contradictions might originate from factors such as study regions, housing conditions, management levels, the total eggs that were weighed, and the duration of the laying period. Furthermore, in the present study, the overall egg weight was dissimilar among stocking densities, which is in line with some authors (Sarica *et al.*, 2008; Zaazaa *et al.*, 2023), but contrary to others (Altan *et al.*, 2002; Erensoy *et al.*, 2021).

The associated changes observed in overall average egg weight with egg-laying time would confirm the findings of some authors (Tůmová *et al.*, 2009; Eleroğlu & Taşdemir, 2020; Eleroğlu, 2021; Krawczyk *et al.*, 2023). It is important to note that the slight deviations in overall egg weight in line with specific egg-laying times are probably related to the total number of egg samples weighed per time, the hen strain, the study period, etc. Moreover, studies by Alig *et al.* (2023a, 2023b) reported increased overall egg weight as hen age increased, which is in line with the present study.

In the current study, the changes identified in the USDA egg size distributions are mostly associated with a biological event, the increase in egg weight that happens with the aging of hens, and consequently the



number of eggs sampled per sub-factor. However, Carey *et al.* (1995) and Al-Rawi *et al.* (1976) found that egg sizes were similar in cages with 6, 8, 12, and 24 hens per cage, and 4, 8, and 12 birds per cage, respectively. Furthermore, these studies explained that similar egg weights among the cage density groups resulted in similar egg sizes. Again, it seems that the source of variation between the findings of the latter studies and the present study might be the difference in cage floor area per bird and the strains that were used.

In the present study, genetic disparity was identified in some egg quality traits, which is in line with some studies (Ketta *et al.*, 2020; Sözcü *et al.*, 2021; Hammershøj *et al.*, 2021; Calik & Obrzut, 2023; Krawczyk *et al.*, 2023; Hejdysz *et al.*, 2023; Tainika *et al.*, 2024c). It is argued that the variations in findings for the specific egg quality traits might be associated with the differences in the strains that were used, study regions, feeding, management, flock age, number of eggs sampled, etc.

Furthermore, stocking density did influence egg weight, shape index, and yolk color. Several authors have also reported stocking density effects on some egg quality traits; for instance, just on shape index (Sarica *et al.*, 2008); and shell-breaking strength (Kang *et al.*, 2016). On the other hand, some studies identified no stocking density impact on any egg quality traits (Şahin *et al.*, 2008; Zaazaa *et al.*, 2023). Generally, there is insufficient literature concerning the influence of stocking density on egg quality traits, warranting further studies.

The current study confirmed age-related changes in egg quality with the aging of hens, as revealed by many previous studies (Şekeroğlu *et al.*, 2014; Samiullah *et al.*, 2017; Yilmaz Dikmen *et al.*, 2017; Eleroğlu & Taşdemir, 2020; Yurtseven *et al.*, 2021; Hammershøj *et al.*, 2021; Şekeroğlu *et al.*, 2024).

Some authors reported genetic disparity regarding meat and blood spots in eggs (Jeffrey, 1945; Campo & Gil, 1998; Hammershøj *et al.*, 2021), agreeing with the current results, but contrary to the study by Lordelo *et al.* (2020). In the present study, the effect of stocking on meat-blood inclusions in albumen is poorly understood, and not in line with Sarica *et al.* (2008), who found similar ratio of eggs with meat and blood inclusions from hens reared at 2000, 1000, 667, and 500 cm² cage floor areas per hen. However, Nalbandov & Card (1944) and Campo & Gil (1998) emphasized that meat and blood inclusions can occur in eggs because of environmental factors that modify fearfulness and stress in birds. Furthermore, Şekeroğlu

et al. (2010) identified increased ratio of eggs with meat and blood spots from hens housed in the cage system as compared to those in deep litter and free-range systems.

Some studies identified a hen age effect on meat-blood inclusions in eggs (Nalbandov & Card, 1944; Jeffrey, 1945; Jensen *et al.*, 1952; Tainika *et al.*, 2024c), contradicting the current study. On the other hand, the present results would be in line with Hammershøj *et al.* (2021), who did not find hen age effect on blood and meat spots in eggs.

The current study confirmed the findings of the previous studies that FCS varies between or among genotypes (Tok *et al.*, 2022; Tainika *et al.*, 2024a). On the other hand, the lower FCS identified in the LSD compared to MSD and HSD groups is not in agreement with various reports, which indicate that FCS decreases when the stocking density is increased (Sarica *et al.*, 2008; Erensoy *et al.*, 2021; Tok *et al.*, 2022).

Furthermore, in the present study, the H/L ratio and TI duration were similar between strains, which is in agreement with Tainika *et al.* (2024a), who identified no genetic effect on TI duration and H/L ratio. What they have in common, is that both studies used Lohmann-related strains, highlighting that the strains might be originating from the same breed and are therefore characterized by similar levels of stress and fear responses, especially when they are housed under similar circumstances.

In addition, it is well known that adequate space allowance is critical for the welfare of birds. As stocking density increases, social stress also increases in birds, which would explain the effect of stocking density on fear and stress responses observed by some studies. For example, Wan *et al.* (2023) identified increased serum corticosterone hormone in birds offered 423 cm² per hen as compared to 564 cm² per hen and 846 cm² per hen. Altan *et al.* (2002) found increased hen rectal temperature in the morning and afternoon at 480 cm² per bird as compared to 640 and 384 cm² per bird. However, this effect was observed in the white strain but not the brown strain, indicating a lack of genetic influence on rectal temperature, which contrasts with the current study.

Furthermore, in the present study, the H/L ratio and TI duration did not vary among stocking densities, taking us back to the argument “exactly how high the stocking density should be for the specific breeds” (Geng *et al.*, 2020) to result in adverse effects on birds’ welfare. Indeed, some reports regarding the effect of stocking density on welfare measures are in line with



the present study. For example, Guo *et al.* (2012) found similar rectal temperatures in hens reared at 398, 543, and 586 cm² per hen. Patterson & Siegel (1998) revealed a similar percentage of heterophils and lymphocytes and the H/L ratio at 142 and 284 cm² cage floor area per hen. Anderson & Adams (1992) reported no difference regarding fearfulness in hens at 221, 249, 277, and 307 cm² per bird. Moreover, in the current study, the stocking density effect on comb temperature, and some blood parameters cannot be fully understood, warranting further studies.

The age effect on FCS was expected based on previous studies (Tok *et al.*, 2022; Tainika *et al.*, 2024a), which reported a decrease in FCS with the aging of birds. Moreover, Tainika *et al.* (2024a) reported the effect of age on some body region temperatures, agreeing with the present findings. The significant interaction effects on feather condition score and some body region temperatures might be linked to the pattern of changes in these variables with the aging of hens.

CONCLUSIONS

This study determined that offering 1016 cm² enriched cage floor area per hen decreased the plumage scores and improved the body weight in hens. However, 762 and 610 cm² cage floor area per hen did not negatively influence other production and welfare variables of hens. It is suggested that the understanding on the effect of enriched cage stocking density on production and welfare traits remains to be refined.

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Author contributions

Conceptualization, AŞ; methodology, AŞ; formal analysis, SHA; investigation, AA, BT, YEŞ, MD, FMG; resources, AŞ; data curation, AA, AŞ; writing—original draft preparation, BT; writing—review and editing, BT, AŞ; visualization, AA; supervision, AŞ, MD; project administration, AŞ, MD; funding acquisition, AŞ. All authors have read and agreed to the published version of the manuscript.

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Data availability statement

The research data will be available upon request.

Conflicts of interest

The authors have no competing interests to declare that are relevant to the content of this article.

Appendix

Not applicable

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