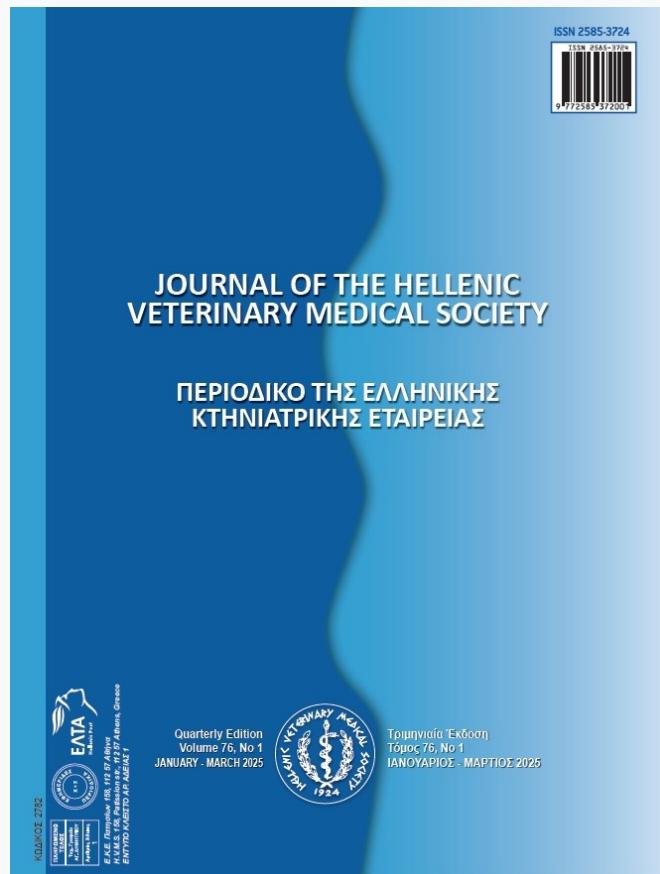


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**Effects of strain, perch and nesting area inclusion or exclusion on performance, egg quality traits, and welfare in laying hens housed in enriched cage system**

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## Effects of strain, perch and nesting area inclusion or exclusion on performance, egg quality traits, and welfare in laying hens housed in enriched cage system

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**ABSTRACT:** The impacts of perch and nesting area inclusion or exclusion on performance, egg quality, and welfare of two laying hen strains were examined. Additionally, the effect of hen age on egg quality traits and some welfare measures was emphasized. Lohmann brown (LB) and Lohmann LSL Classic (LW) hens were randomly allotted to cage treatments according to a 2 perch (with; PYES vs. without; PNO perch) by 2 nesting area (with; NYES vs. without; NNO nesting area), with four replicates per treatment, each with 20 hens, commencing and ending at 20 and 52 weeks of hen age, respectively. Live body weight, age at 50% egg production, hen day and hen house egg production, livability, egg quality traits, overall egg weight, duration of tonic immobility, blood parameters, feather condition score, and body region temperature were assessed. From the results, LB hens were heavier and had a higher comb, breast region, and footpad surface temperature than LW hens ( $P<0.01$ ). PNO housed hens were heavier at 50% egg production than PYES housed hens however; eggs with meat and blood spots in albumen were higher in PYES than in PNO housed hens ( $P<0.05$ ). In addition, PYES housed hens had a higher footpad surface temperature than those housed in PNO cages. NNO housed hens reached 50% egg production earlier, had a higher comb and rectal temperature, and better feather scores compared to NYES housed hens ( $P<0.05$ ;  $P<0.01$ ). NYES housed hens produced eggs with a darker yolk color than NNO housed hens ( $P<0.05$ ). Furthermore, the nesting area effect on duration of tonic immobility approached a significant level ( $P=0.054$ ), with a shorter duration for NYES than NNO housed hens. Age effect was observed on average egg weight, and egg quality traits apart from shape index, meat-blood spots in the yolk, feather score and body region temperatures ( $P<0.01$ ), and egg-laying time impacted average egg weight ( $P<0.01$ ). The study suggests no substantial evidence that the exclusion of a perch and nesting area in the enriched cages compromises performance and welfare measures in hens in addition to relatively slight differences between the strains.

**Keywords:** Egg quality; Enriched cage system; Genotype; Laying hen; Production performance; Welfare

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## INTRODUCTION

The worldwide implementation of enriched cages occurred after the ban on conventional cages in the European Union (EU) in 2012 (Directive EU, 1999). Generally, enriched cages are designed to offer at least 750 cm<sup>2</sup> of cage area/hen and structural elements including perches, nesting area, and scratching area. The above-outlined features would ensure that birds have the potential to express natural behaviors including nesting, roosting, scratching, and stretching. Furthermore, the development of enriched cages was an effort to enhance the welfare of hens housed in cage systems (Lay et al., 2011; Tainika and Şekeroglu, 2020).

Understanding the performance and welfare of hens and their evaluation warrants the utilization of several indicators, especially where inter and intra-strain differences occur in the manner the hens adapt to the housing environment. Indeed, some authors have reported strain differences in some performance traits, for instance, hen day egg production (Ketta et al., 2020; Rakonjac et al., 2021; Sharma et al., 2022), and mortality (Rakonjac et al., 2021). Genetic structure affects egg quality traits; egg weight, shell breaking strength, shell thickness, albumen pH, and Haugh unit (Krawczyk et al., 2023), heterophil-to-lymphocyte (H/L) ratio as an indicator of stress (Hassan et al., 2023), some body region temperatures (Tainika et al., 2024a), feather condition score (Tok et al., 2022; Tainika et al., 2024a), and duration of tonic immobility (Wei et al., 2022).

Although enriched cages are recognized for their attempt to improve the welfare of hens, there is a scarcity of literature and clear evidence that excluding structural elements in cages would compromise the performance and welfare of hens. For instance, Barnett et al. (2009) found significantly increased feather damage but better foot condition for hens in cages with a perch compared to those without a perch. However, the latter authors observed no differences in egg laying, hen day egg production, body weight, and white blood cell count between hens in cages with or without perches. Additionally, housing hens in cages with or without a nest box did not influence the production and welfare traits aside from egg-laying behaviors. Furthermore, Engel et al. (2019) identified higher feather loss in hens in cages with access to a nest box at only 34 weeks of age but no difference in H/L ratio, egg weight, and hen day egg production between hens in cages with or without access to

a nest box. It is noted that studies by Barnett et al. (2009) and Engel et al. (2019) did not highlight clear or meaningful evidence that the lack of a perch or a nest box in cages implicates the welfare of hens.

Meanwhile, the age of hens is another factor that can influence egg quality traits (Samiullah et al., 2014; Samiullah et al., 2017; Yılmaz Dikmen et al., 2017; Yurtseven et al., 2021; Calik and Obrzut, 2023; Şekeroglu et al., 2024; Tainika et al., 2024c). Also, some previous reports have indicated the influence of egg-laying time on egg weight. For instance, Krawczyk et al. (2023) identified decreased egg weight in eggs collected from 7:00 compared to 13:00. This would contrast with Tůmová et al. (2009), who had earlier reported that the heaviest eggs were obtained from 06:00 compared to 10:00, and 14:00.

Generally, before and following the ban on conventional cages, there was considerable support for enriched cage design particularly in Europe, associated with their ability to increase the expression of natural behaviors in laying hens. Apparently, there is an important discussion “end of cage hen” in the EU because welfare groups do not accept that there is improved welfare of hens in cages even with furniture items. While most studies have focused on aspects associated with furniture items, for instance, perch dimension, material, and design (Bist et al., 2023) and their ability to permit the associated behavioral repertoire in cages, there remains a research or literature gap regarding the economic benefits (production performance and egg quality of hens) of the presence of furniture items either alone and in combination, warranting in-depth studies. It is argued that the transfer of hens from cages with furniture items, that is, perch and nesting area to cages without both or one furniture item would modulate the biological systems or functioning associated with changes in performance, egg quality traits, and welfare. In addition, studies on furniture items used a single strain of hens however, genotype differences in body region temperature, stress, and fear responses can occur as hens attempt to cope with the housing environment. Consequently, the well-known production parameters, that is, live body weight, livability, egg production, and feather condition of hens might be adversely influenced by the underlying physiological responses as strains attempt to cope.

Therefore, this study assessed the effect of strain and inclusion or exclusion of a perch or nesting area on the performance, egg quality, and welfare of hens

housed in the enriched cage system. Additionally, the study examined the hen age effect on egg quality and welfare traits such as body region temperature and feather score.

## MATERIALS AND METHODS

This study was carried out at Niğde Ömer Halisdemir University, Ayhan Şahenk Agricultural Application and Research Center. A total of 640 birds, 320 birds of each of Lohmann brown (LB) and Lohmann LSL Classic (LW) were used as animal materials. It is worth noting that the birds used in this study were purchased from a commercial breeder firm at 16 weeks (wk) of age. When at the commercial unit of the university, the birds were randomly placed in enriched cages, before the study was set up after four weeks. The enriched cage system offered 8 stainless drinking nipples that were shared between back-to-back cages, and a feeder trough in front of the cages. Water and feed were provided *ad libitum*. The cage floor was made of wire mesh. Each cage unit was 240 cm wide, 63.5 cm deep, and 60 cm high, and equipped with a dark blue curtained nesting area of 40 cm × 33.5 cm × 30 cm: length × width × height. In addition, there were two horizontal perches, each of length 180 cm with a nail shortener attached to each, and a scratch-pad.

The cages were in an environmentally controlled barn for temperature, light, and ventilation. The lighting program followed the Lohmann breeder management guide for alternative housing (Lohmann, 2021)

and lighting was provided by warm white LED bulbs of 24-watts, controlled automatically. In addition, the regulatory requirements and the standard recommendations for the region towards vaccination were followed. During this period, the management of birds followed the standards of the Lohmann Breeder Company.

## Experimental treatment cages

The experimental design involved treatments that were allotted to enriched cage units that were positioned facing the wall of the barn. At 20 wk of age, the onset of the study, birds were randomly distributed to treatment cages in compliance with a 2 perch (with perch, PYES vs. without perch, PNO) by 2 nesting area (with nesting area, NYES vs. without nesting area, NNO) factorial design. This experimental design offered four replicates for each treatment and each strain, comprising 20 hens per replicate treatment cage. The birds were within similar live body weight ranges and feather condition scores at the beginning of the study for each of the subgroups. The placement body weights at 20 wk of age were 1125.9 g and 1438.4 g for LW and LB strains, respectively, and 1273.1 g and 1291.2 for PYES and PNO cages, respectively. It was 1273.2 g and 1291.1 g for NYES and NNO cages, respectively. The floor area allocated per animal was determined as 762 cm<sup>2</sup>. The study duration was 32 wk, from 20 to 52 wk of hen age.

The feed given to the birds before and during the study is indicated in Table 1.

**Table 1.** Composition and ingredients of commercial diet that was used at various ages during the study.

Nutrient composition	Type of feed (age of hens)		
	Pre-peak lay (16-23 weeks)	Layer 1. phase (23-33 weeks)	Layer 2. phase (34 weeks until the end of the study)
Crude protein, %	17.5	17	15.61
Crude cellulose, %	3.6	4.5	4.8
Crude ash, %	13.6	13.7	12.2
Crude fat, %	4.4	4.9	3.83
Calcium, %	3.9	3.9	3.83
Phosphorous, %	0.4	0.4	0.42
Sodium, %	0.1	0.1	0.16
Lysine, %	0.8	0.8	0.76
Methionine, %	0.4	0.4	0.37
Metabolizable energy, Kcal/Kg	2700	2700	2700

Ingredients: Maize (produced from genetically modified maize), soya bean meal (produced from genetically modified soya), wheat, calcium carbonate, sunflower seed meal, Dried distillers grains (DDGS: produced from genetically modified maize), soya oil, dicalcium phosphate, sodium chloride, sodium bicarbonate; Vitamin A 12.000 IU; Vitamin D3 2.400 IU; Vitamin E 30 Mg / Kg; Mg 80 mg; Zn 60 mg; Cu 5 mg; Fe 60 mg; I 2 mg; Se 0.15 mg; Co 0.5 mg

## Data collection

### Performance traits

During the study, layer hybrids in each replicate cage unit were individually weighed with a scale of 0.1 g weighing precision at placement; 20 wk of age, age at 50% egg production, and on the final day of the study. Later, the average live hen body weights per cage unit were determined.

From the day of the first egg, egg yield per replicate cage unit was recorded daily, and the weights of individual eggs were determined weekly.

Age at 50% egg production was determined as the day 50% of eggs were obtained from each treatment cage unit. Egg yield data was used to calculate hen house and hen day egg production (HHE and HDE, respectively) for each treatment group using the formulas below.

$$HDE = \left( \frac{\text{Number of eggs produced during the period}}{\text{Number of hen days in the period}} \right) \times \text{days}$$

$$HHE = \left( \frac{\text{Number of eggs produced during the period}}{\text{Number of hens present at that period}} \right)$$

Additionally, egg weights were taken with a digital scale of 0.01 g weighing precision based on egg-laying time, that is, 8:30 a.m., 12:00, and 3.30 p.m. Subsequently, the average egg weights at the end of the study were determined.

Dead birds were recorded daily per treatment cage unit, and the data was used to calculate livability in percentage as shown in the formula below.

Livability = (Number of hens at the beginning of the study - number of hens remaining at the end of the study) / (number of hens at the beginning of the study)) × 100.

### Egg quality traits

96 eggs (48 eggs per strain; 3 eggs from each treatment replicate) were taken to the laboratory every 4 weeks between 32 and 52 wk of age, however, only 64 eggs (32 eggs per strain; 2 eggs from each treatment replicate) were always analyzed. Quality analyses were performed after the eggs were held at room temperature for a day.

A scale of 0.01 g weighing precision was used to determine egg weight (g). Afterward, a digital caliper (0.01 mm) was used to obtain values for the egg

length and width to be used to calculate the shape index (SI) as indicated below.

$$SI, \% = \frac{Egg\ width\ h}{Egg\ length\ h} \times 100$$

The egg was then subjected to an Orka Food Technology egg force reader, Israel, which reads in "Kg. f" to record the shell-breaking strength. Later, a manual micrometer was used to measure albumen and yolk heights of the broken egg on a glass table. At the same time, a digital caliper (0.01 mm) was utilized to measure albumen length and width and yolk diameter. Also, the color of the yolk was scored based on a DSM yolk color fan and a manual pH meter was used to determine the albumen pH.

Additionally, some of the above data was later used to calculate the egg surface area (ESA), albumen index (AI), yolk index (YI), and Haugh unit (HU) as indicated below.

$$ESA, (\text{cm}^2) = 3.9782 \times \text{egg weight in grams}^{0.70}, \text{ according to Carter (1975).}$$

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

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

$$HU = 100 \log (\text{albumen height} - 1.7 \text{ egg weight}^{0.37} + 7.57), \text{ according to Haugh (1937).}$$

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

Shell thickness (mm) was determined as the average shell thickness values taken from three different regions of an egg, using a metrica manual micrometer (0.01mm). The shell thickness at the blunt, center, and pointed portions of eggs were measured on shells without the shell membrane.

### Welfare traits

Welfare measures were assessed from 64 hens (32 hens from each strain; 2 hens per replicate treatment) randomly selected from each replicate treatment cage. The traits involved in the study included the following.

**Duration of tonic immobility (TI, seconds):** One bird at a time was captured and transferred to an empty room in the same barn and tested for the duration of TI as described by Jones and Faure (1981). Briefly, in the testing room, the bird was held on her back in the rectangular wooden curdle. One hand of the assessor was placed on the breast region and the other covered the hanging head of the bird. 15 seconds were counted and thereafter, the assessor removed his hands slowly from the bird. The assessor sat approximately one meter from the bird but maintained direct eye contact with the bird. Immediately after the withdrawal of hands, the stop clock was started, and the time taken by the hen to return to the normal position was recorded as its duration of TI in seconds. If the hen did not right 10 minutes after the TI induction, 600 seconds were given for the hen. Importantly, if the bird returned to the right position in less than 10 seconds after the withdrawal of hands, TI induction was repeated however for a maximum of three occasions. Beyond three times of TI induction, TI was deemed unsuccessful, and a score of 0 seconds was recorded. It should be noted that testing for TI was performed by a single Ph.D. researcher experienced in poultry welfare and behavior at 52 weeks of age.

**Blood parameters (%):** Blood collection was performed after birds had been assessed for TI at 52 weeks of age. Whole blood was collected from the wing vein using a 2-cc sterile syringe. A drop of blood was then put on a base or smear slide which was run by another slide to draw a thin blood smear. The smear blood slides were then transported to the laboratory and the number of 100 blood leukocytes (lymphocytes, monocytes, heterophil, eosinophils, and basophils) were counted under a microscope after the staining process involving May-Grunwald and Giemsa stains. The number of heterophils (H) and lymphocyte (L) cells for each bird was used to determine the H/L ratios according to Gross and Siegel (1983).

**Feather condition scores:** Feather condition was evaluated using a 4-point scoring system for the individual region of the hen: neck, back, cloaca, tail, wing, and breast. The methodology applied was based on Tauson et al. (2005). Later, the individual body region scores were summed together to obtain the total feather score for the individual hen. Scores 24 and 6 indicated very good and poor feather conditions, respectively. Scoring of feather condition was performed between 29 and 52 weeks of hen age at an interval of 4 weeks.

**Body region temperatures (°C):** Rectal temperature was measured by a digital thermometer (MEDIX KD-106, China) kept in the cloaca of hens until the temperature rise stabilized. The infrared thermometer (LOYKA DARK II, China) was used to measure the breast region, comb, and footpad surface temperatures of the hens. Assessment of body temperatures was performed each time the feather condition of hens was scored.

**Footpad dermatitis** was determined using a 4-point scale as indicated by the Welfare Quality Assessment Protocol (2009).

### Statistical analysis

In the study, the variance homogeneity test was examined with the Levene test and it was determined that the variances of the data were homogeneous. For this reason, analysis of variance was used to analyze the data. Although it varies depending on the variables examined, the following model was generally used.

$$Y_{ijklm} = \mu + Y_i + G_j + T_k + F_l + YG_{ij} + YT_{ik} + YF_{il} + \dot{Y}_{jk} + GF_{jk} + TF_{kl} + YGT_{ijk} + YGF_{ijl} + YTF_{ikl} + GTF_{jkl} + YGTF_{ijkl} + e_{ijklm}$$

In the equation:  $Y_{ijklm}$  : observation value,  $\mu$  : Population mean  $Y_i$ : i. age effect (week),  $G_j$ : j. strain effect,  $T_k$ : k. perch effect,  $F_l$ : l. nesting area effect  $YG_{ij}$ ;  $YT_{ik}$ ;  $YF_{il}$ ;  $\dot{Y}_{jk}$ ;  $GF_{jk}$ ;  $TF_{kl}$ ;  $YGT_{ijk}$ ;  $YGF_{ijl}$ ;  $YTF_{ikl}$ ;  $GTF_{jkl}$ ;  $YGTF_{ijkl}$  : interaction effects, and  $e_{ijklm}$ : random error.

Age effect and interaction effects with other factors were included only for egg quality, feather score, and body temperatures. Strain, perch, and nesting area effects and interactions between these factors were examined in live weight, tonic immobility, and livability variables.

The following model was used to determine the environmental factors affecting egg weight.

$$Y_{ijklmn} = \mu + Y_i + G_j + T_k + F_l + S_m + e_{ijklmn}$$

In the equation,  $Y_{ijklmn}$ : observation value,  $\mu$  : population mean,  $Y_i$ : i. age effect (week),  $G_j$ : j. strain effect,  $T_k$ : k. perch effect,  $F_l$ : l. nesting area effect,  $S_m$ : m. egg-laying time,  $e_{ijklmn}$ : random error. General Linear Model procedure was applied to analyze the model. Mean and standard error values are given for descriptive statistics values. Duncan's multiple comparison test was used to determine differences between groups at  $P < 0.05$ . Whether the presence of

blood and meat stains in albumen and yolks was dependent on hen age, strain, and the inclusion or exclusion of perch or nesting site was determined by Pearson chi-square analysis. IBM SPSS 21 package program was used in the analysis of all statistical methods (IBM Corp, 2012).

## RESULTS

### Performance traits

The results of this study showed that LB were heavier than LW hens across all the sampled weeks ( $P<0.001$ ; Table 2). However, there was no strain ef-

fect on age at 50% egg production, livability, and hen day and hen house egg production ( $P>0.05$ ). Hens reared in PNO cages were heavier than those in PYES cages at age at 50% egg production ( $P<0.05$ ; Table 2). Furthermore, age at 50% egg production was earlier for hens reared in NNO cages than in NYES cages ( $P<0.05$ ; Table 2). In contrast, hen weights at 52 wk of age, livability, and hen day and hen house egg production were not different between hens in cages with or without a perch and nesting area ( $P>0.05$ ).

There were significant interaction effects between strain  $\times$  perch for livability, and hen house egg produc-

**Table 2.** Impact of strain, inclusion or exclusion of perch or nesting area on live hen weights, age at 50% egg production, livability, and egg production

Factor		HW at 50% egg production (g)	HW at 52 weeks of age (g)	Age at 50% egg production (days)	Livability (%)	Hen day egg production (number of eggs)	Hen house egg production (number of eggs)
Strain (S)	LW	1477.4	1532.9	159.6	94.375	187.08	184.23
	LB	1870.8	1856.9	160.7	95.625	188.67	186.53
Perch (P)	PYES	1659.3	1697.4	159.9	94.063	188.10	184.78
	PNO	1688.8	1695.1	160.4	95.938	187.65	185.98
Nesting area (N)	NYES	1683.1	1698.9	161.3	96.563	188.77	186.95
	NNO	1665.1	1693.5	159.0	93.438	186.98	183.81
SEM		9.107	9.063	0.453	0.979	1.664	1.775
P value							
S		<0.001	<0.001	0.187	0.452	0.652	0.511
P		<0.002	0.698	0.551	0.262	0.897	0.732
N		0.055	0.635	<0.012	0.068	0.612	0.373
S $\times$ P		0.843	0.335	0.301	<0.013	0.151	<0.047
S $\times$ N		<0.004	0.922	0.187	0.705	0.494	0.554
P $\times$ N		0.961	0.399	0.551	0.139	0.905	0.771
S $\times$ P $\times$ N		0.317	0.893	0.655	0.031	0.265	0.129

Abbreviations: HW; live hen weight, LW; Lohmann LSL Classic, LB; Lohmann brown; SEM; standard error of mean,  $\times$ : interaction between different factors,

Significant difference between means at  $P<0.05$ .

**Table 3.** Impact of hen age, strain, inclusion or exclusion of perch or nesting area, and egg-laying time on overall average egg weight

Factor		N	Egg weight (g)	P
Hen age, week	20-32	5036	58.85 <sup>c</sup>	<0.001
	33-42	5740	63.25 <sup>b</sup>	
	43-52	5435	63.74 <sup>a</sup>	
Strain	Lohmann White	8169	61.68	<0.001
	Lohmann Brown	8042	62.42	
Perch	PYES	8097	61.86	<0.001
	PNO	8114	62.24	
Nesting area	NYES	8311	62.03	0.927
	NNO	7900	62.06	
Egg-laying time	9:00 a.m.	6032	61.97 <sup>b</sup>	<0.001
	12:00 noon	8601	62.34 <sup>a</sup>	
	3:00 p.m.	1578	60.72 <sup>c</sup>	
SEM			0.042	

Abbreviations: SEM; standard error of mean, n; number of eggs. Means within the column with different letter superscripts differ significantly ( $P<0.05$ ).

tion ( $P<0.05$ ; Table 2). Also, the interaction between strain  $\times$  nesting area and strain  $\times$  perch  $\times$  nesting area influenced hen weight at age at 50% egg production ( $P<0.05$ ).

### Overall average egg weight

Table 3 indicates results for overall average egg weight during the study. Heavier eggs were obtained from LB than LW strains, and in hens reared in PNO cages than those in PYES cages ( $P<0.001$ ). There was no significant effect of nesting area treatment on average egg weight ( $P>0.05$ ). However, average egg weight increased as the hen age increased ( $P<0.001$ ). Furthermore, heavier eggs were obtained at 12:00 a.m. than at 9:00 a.m., which was higher than at 3:00 p.m. ( $P<0.001$ ).

### Egg quality traits

Table 4 indicates results for egg quality traits. There was no effect of strain, perch, and nesting area on egg quality traits ( $P>0.05$ ) aside from yolk color

score, which was influenced by nesting area treatment ( $P<0.05$ ). Eggs with darker yolks were obtained from hens reared in NYES cages than in NNO cages.

Excluding shape index, the remaining egg quality traits varied as hen age increased ( $P<0.001$ ); the heaviest and lightest eggs were obtained at 52 and 43 wk of age, respectively. Eggs with the strongest and thickest shells were at 43 wk of age and 43 and 47 wk of age, respectively. The albumen index was highest and lowest at 34 and 47 wk of age and 39 wk of age, respectively. At 39 wk of age and 34 and 47 wk, the Haugh unit was lowest and highest, respectively. The Yolk index was higher at 34 wk of age than other weeks which was similar. Eggs with more dark yolks were observed at 34 wk and the highest albumen pH was identified in eggs at 52 wk of age.

There was a significant interaction effect of age  $\times$  strain for shell thickness and yolk color score ( $P<0.05$ ;  $P<0.001$ ), age  $\times$  perch for yolk index ( $P<0.05$ ), and

**Table 4.** Impact of hen age, strain, inclusion or exclusion of perch or nesting area on egg quality traits

Factor	n	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, wk)	34	64	64.54 <sup>b</sup>	77.92	5.276 <sup>b</sup>	0.410 <sup>a</sup>	12.95 <sup>a</sup>	97.61 <sup>cd</sup>	45.82 <sup>a</sup>	12.98 <sup>a</sup>
	39	64	65.48 <sup>ab</sup>	77.14	5.080 <sup>b</sup>	0.396 <sup>b</sup>	8.50 <sup>d</sup>	80.30 <sup>a</sup>	39.68 <sup>b</sup>	11.08 <sup>d</sup>
	43	64	62.51 <sup>c</sup>	77.86	5.559 <sup>a</sup>	0.412 <sup>a</sup>	9.90 <sup>c</sup>	88.23 <sup>b</sup>	40.39 <sup>b</sup>	11.14 <sup>d</sup>
	47	64	65.43 <sup>ab</sup>	78.17	5.156 <sup>b</sup>	0.413 <sup>a</sup>	12.95 <sup>a</sup>	100.37 <sup>d</sup>	40.48 <sup>b</sup>	11.48 <sup>c</sup>
	52	64	66.40 <sup>a</sup>	77.15	5.076 <sup>b</sup>	0.396 <sup>b</sup>	11.60 <sup>b</sup>	96.58 <sup>c</sup>	40.62 <sup>b</sup>	11.89 <sup>b</sup>
Strain (S)	LW	160	64.79	77.85	5.180	0.404	11.21	93.42	41.34	11.76
	LB	160	64.95	77.45	5.279	0.407	11.15	91.82	41.45	11.68
Perch (P)	PYES	160	64.57	77.64	5.252	0.404	11.10	92.68	41.63	11.73
	PNO	160	65.18	77.65	5.207	0.406	11.26	92.56	41.16	11.71
Nesting area (N)	NYES	160	65.06	77.81	5.249	0.407	11.05	91.84	41.13	11.81
	NNO	160	64.68	77.49	5.210	0.403	11.31	93.40	41.67	11.63
SEM		0.201	0.173	0.041	0.001	0.141	0.615	0.219	0.055	0.016
P value										
A		<0.001	0.180	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
S		0.669	0.238	0.227	0.094	0.776	0.075	0.750	0.288	0.054
P		0.105	0.982	0.579	0.364	0.399	0.896	0.162	0.806	0.604
N		0.312	0.342	0.631	0.074	0.194	0.081	0.113	<0.018	0.060
A×S		0.87	0.574	0.440	<0.010	0.318	0.137	<0.002	0.246	0.067
A×P		0.196	0.063	0.552	0.162	0.861	0.462	<0.041	0.836	0.949
A×N		0.899	<0.029	0.451	0.749	0.409	0.166	<0.001	0.384	<0.031
S×P		0.055	0.844	0.433	0.405	<0.007	0.146	0.536	<0.028	<0.039
S×N		0.163	0.257	0.266	0.874	0.994	0.871	0.146	<0.018	0.658
P×N		0.776	0.860	0.457	0.799	0.099	0.788	0.646	0.369	0.704
A×S×P		0.281	0.189	0.384	0.977	0.242	0.157	0.451	0.466	0.550
A×S×N		0.992	0.055	0.480	0.500	0.251	0.620	0.320	0.088	0.213
A×P×N		0.677	0.144	0.907	<0.048	<0.036	0.423	0.107	0.292	0.765
S×P×N		0.463	0.913	0.876	<0.001	<0.001	0.089	<0.013	0.121	0.614
A×S×P×N		0.016	0.615	0.388	0.192	0.255	0.065	0.572	0.952	0.396

Abbreviations: LW; Lohmann LSL Classic, LB; Lohmann brown; SEM; standard error of mean,  $\times$ : interaction between different factors, temp: temperature, kg. f: kg. force (kilogram-force), n; number of eggs. Means within the column with different letter superscripts differ significantly ( $P<0.05$ ).

age  $\times$  nesting area for shape index ( $P<0.05$ ), yolk index ( $P<0.001$ ), and albumen pH ( $P<0.05$ ). Additionally, there was a significant interaction effect of strain  $\times$  perch for albumen index, yolk color score, and albumen pH ( $P<0.001$ ;  $P<0.05$ ), and strain  $\times$  nesting area for yolk color ( $P<0.05$ ). In addition, there was a significant interaction effect of age  $\times$  perch  $\times$  nesting area for shell thickness and albumen index, and strain  $\times$  perch  $\times$  nesting area for shell thickness, albumen index, and yolk index.

The percentage of eggs with blood-meat spots in the albumen was not different between strains, and nesting area treatment ( $P>0.05$ ; Table 5). However, perch influenced the percentage of eggs with blood-meat spots in the albumen ( $P<0.05$ ); more eggs with blood spots in albumen were identified from PYES cages, and more eggs with meat spots in albumen were observed from PNO cages. Furthermore, the effect of age on meat-blood spots in albumen nearly approached a significant level ( $P = 0.064$ ).

The percentage of eggs with blood-meat spots in

the yolk did not differ between strains, perch, and nesting area treatment ( $P>0.05$ ; Table 6). However, there was an effect of hen age on the percentage of eggs with blood-meat spots in the yolk ( $P<0.001$ ); the percentage of eggs with blood spots was highest and lowest at 52 wk and 43 and 47 wk of age, respectively. Additionally, the percentage of eggs with meat spots in the yolk was higher at 47 wk of age.

### Welfare traits

The results for blood parameters are shown in Table 7. There was no effect of strain, perch, nesting area, and their interactions on the blood parameters ( $P>0.05$ ).

Table 8 indicates results for total feather condition scores and body region temperatures. While feather condition score and rectal temperature were not different between strains ( $P>0.05$ ), the comb, breast region, and footpad surface temperatures differed ( $P<0.001$ ). All the body surface temperatures were higher for the LB than the LW strain. There was a perch effect on only footpad surface temperature ( $P<0.001$ ), higher

**Table 5.** Impact of hen age, strain, inclusion or exclusion of perch or nesting area on presence of blood and meat spots in egg albumen

Factor	Eggs with or without blood-meat spots in albumen			Total	$\chi^2$	P value	
	Normal	Blood spots	Meat spots				
Hen age, wk	34	62 (96.9%)	1 (1.6%)	1 (1.6%)	64 (100%)	14.770	0.064
	39	60 (93.8%)	2 (3.1%)	2 (3.1%)	64 (100%)		
	43	59 (92.2%)	3 (4.7%)	2 (3.1%)	64 (100%)		
	47	55 (85.9%)	9 (14.1%)	0 (0%)	64 (100%)		
	52	54 (84.4%)	7 (10.9%)	3 (4.7%)	64 (100%)		
	LW	145 (90.6%)	10 (6.3%)	5 (3.1%)	160 (100%)		
Strain	LB	145 (90.6%)	12 (7.5%)	3 (1.9%)	160 (100%)	0.682	0.711
	PNO	145 (90.6%)	8 (5%)	7 (4.4%)	160 (100%)		
	PYES	145 (90.6%)	14 (8.8%)	1 (0.6%)	160 (100%)		
Perch	NNO	143 (89.4%)	12 (7.5%)	5 (3.1%)	160 (100%)	6.136	<0.047
	NYES	147 (91.9%)	10 (6.3%)	3 (1.9%)	160 (100%)		
Total		290 (90.6%)	22 (6.9%)	8 (2.5%)	320 (100%)	0.737	0.692

Abbreviations: LW; Lohmann LSL Classic, LB; Lohmann brown. Significant difference at  $P<0.05$ . The first value in front of the bracket is the absolute number of eggs.

**Table 6.** Impact of hen age, strain, inclusion or exclusion of perch or nesting area on presence of blood and meat spots in the yolk

Factor		Eggs with or without blood-meat spots in the yolk			Total	$\chi^2$	P
		Normal	Blood spots	Meat spots			
Age, wk	34	56 (87.5%)	8 (12.5%)	0 (0%)	64 (100%)	27.341	<0.001
	39	57 (89.1%)	7 (10.9%)	0 (0%)	64 (100%)		
	43	58 (90.6%)	5 (7.8%)	1 (1.6%)	64 (100%)		
	47	55 (85.9%)	5 (7.8%)	4 (6.3%)	64 (100%)		
	52	46 (71.9%)	18 (28.1%)	0 (0%)	64 (100%)		
	LW	137 (85.6%)	22 (13.8%)	1 (0.6%)	160 (100%)		
Strain	LB	135 (84.4%)	21 (13.1%)	4 (2.5%)	160 (100%)	1.838	0.399
	PNO	133 (83.1%)	24 (15%)	3 (1.9%)	160 (100%)		
	PYES	139 (86.9%)	19 (11.9%)	2 (1.3%)	160 (100%)		
Perch	NNO	134 (83.8%)	24 (15%)	2 (1.3%)	160 (100%)	0.914	0.633
	NYES	138 (86.3%)	19 (11.9%)	3 (1.9%)	160 (100%)		
Total		272 (85%)	43 (13.4%)	5 (1.6%)	320 (100%)	0.840	0.657

Abbreviations: LW; Lohmann LSL Classic, LB; Lohmann brown. Significant difference at P<0.05. The first value in front of the bracket is the absolute number of eggs.

**Table 7.** Impact of strain, inclusion or exclusion of perch or nesting area on blood parameters and duration of tonic immobility (TI)

Factor	n	Lymphocyte (%)	Monocyte (%)	Heterophil (%)	Eosinophil (%)	Basophil (%)	H/L ratio	TI (seconds)
Strain	LW	32	45.03	22.41	27.63	4.19	0.750	0.632
(S)	LB	32	44.56	22.38	28.44	3.81	0.813	0.665
Perch (P)	PYES	32	43.88	22.47	28.97	4.03	0.656	0.685
	PNO	32	45.72	22.31	27.09	3.97	0.906	0.611
Nesting area (N)	NYES	32	44.03	21.91	29.19	4.00	0.875	0.688
	NNO	32	45.56	22.88	26.88	4.00	0.688	0.609
SEM		0.74	0.48	0.72	0.30	0.110	0.025	25.978
P value								
S		0.758	0.975	0.567	0.544	0.781	0.511	0.822
P		0.229	0.874	0.189	0.919	0.268	0.146	0.346
N		0.316	0.327	0.107	0.999	0.405	0.121	0.054
S×P		0.499	0.975	0.597	0.364	0.268	0.678	0.634
S×N		0.580	0.727	0.895	0.419	0.405	0.746	0.419
P×N		0.245	0.547	0.220	0.613	0.268	0.244	0.152
S×P×N		0.918	0.157	0.138	0.364	0.999	0.209	0.194

Abbreviations: LW; Lohmann LSL Classic, LB; Lohmann brown; SEM; standard error of mean, n; number of birds, ×: interaction between different factors. Significant difference at P<0.05.

**Table 8.** Effect of hen age, strain, inclusion or exclusion of perch or nesting area on total feather score and body region temperatures

Factor		N	Feather score	n	Comb temp (°C)	Breast region temp (°C)	Footpad surface temp (°C)	Rectal temp (°C)
Age (A, wk)	29	64	23.95 <sup>a</sup>	-	-	-	-	-
	33	64	23.94 <sup>a</sup>	64	35.73 <sup>a</sup>	35.61 <sup>c</sup>	35.01 <sup>a</sup>	40.65 <sup>c</sup>
	37	64	23.94 <sup>a</sup>	64	35.75 <sup>a</sup>	35.60 <sup>c</sup>	34.77 <sup>a</sup>	40.83 <sup>b</sup>
	41	64	20.70 <sup>c</sup>	64	35.52 <sup>a</sup>	35.92 <sup>c</sup>	34.23 <sup>a</sup>	40.60 <sup>c</sup>
	45	64	22.28 <sup>b</sup>	64	34.86 <sup>a</sup>	39.42 <sup>a</sup>	33.94 <sup>a</sup>	40.59 <sup>c</sup>
	52	64	19.22 <sup>d</sup>	64	25.72 <sup>b</sup>	38.25 <sup>b</sup>	26.03 <sup>b</sup>	41.20 <sup>a</sup>
Strain (S)	LW	192	22.31	160	32.80	36.61	32.22	40.80
	LB	192	22.36	160	34.24	37.30	33.37	40.75
Perch (P)	PYES	192	22.40	160	33.74	37.03	33.33	40.74
	PNO	192	22.28	160	33.30	36.88	32.27	40.81
Nesting area (N)	NYES	192	22.14	160	32.93	36.84	32.74	40.69
	NNO	192	22.54	160	34.11	37.08	32.85	40.85
SEM			0.111		0.278	0.143	0.263	0.031
P value								
A			<0.001		<0.001	<0.001	<0.001	<0.001
S			0.652		<0.001	<0.002	<0.001	0.396
P			0.321		0.176	0.495	<0.002	0.212
N			<0.001		<0.001	0.278	0.759	<0.005
A×S			0.435		0.107	<0.019	0.558	0.897
A×P			0.492		0.037	0.720	0.257	0.544
A×N			<0.001		0.565	0.646	0.550	0.093
S×P			0.787		0.667	0.535	<0.001	0.121
S×N			0.149		0.261	0.176	<0.026	0.565
P×N			0.072		0.339	0.853	0.698	0.631
A×S×P			0.998		0.667	0.590	<0.003	0.710
A×S×N			0.814		0.890	0.310	0.745	0.845
A×P×N			0.703		0.801	0.356	0.928	0.646
S×P×N			0.105		0.851	0.118	<0.033	0.319
A×S×P×N			0.282		0.404	0.565	0.056	0.612

Abbreviations: LW; Lohmann LSL Classic, LB; Lohmann brown; SEM; standard error of mean, n; number of birds, ×: interaction between different factors, temp: temperature. Means within the column with different letter superscripts differ significantly ( $P<0.05$ ). Footpad dermatitis was not observed in the study.

for hens in PYES cages than in PNO cages. Furthermore, the nesting area effect was observed on total feather condition score, comb, and rectal temperatures, all higher in hens reared in NNO cages than in NYES cages ( $P<0.001$ ).

Also, the age effect was identified on total feather condition score, and comb and footpad temperatures, showing a decreasing trend as hen age increased ( $P<0.001$ ). However, there was no clear trend for the age effect on breast region and rectal temperature; breast temperature was highest and lowest at 45 wk and from 33 to 41 wk of age, respectively. Rectal temperature increased at 37 wk, followed by a decreasing trend onwards.

There was a significant interaction effect of age

× strain for breast region temperature ( $P<0.05$ ), age × nesting area for feather condition score ( $P<0.001$ ), strain × perch and strain × nesting area for footpad temperature ( $P<0.001$ ;  $P<0.05$ ). Also, there was a significant interaction effect of age × strain × perch and strain × perch × nesting area for footpad surface temperature ( $P<0.001$ ;  $P<0.05$ ).

## DISCUSSION

In this study, although the live body weight was dissimilar between strains and is consistent with the Lohmann management guide, the figures for each strain were not in the range of the industry targets (Lohmann, 2021). In the guide, for instance, at 52 wk of age, the weight range is reported as between 1916 - 2034 g and 1685 - 1825 g for Lohmann brown

and Lohmann LSL Classic, respectively. Age at 50% egg production did not differ between strains but was similar to the industry targets for both strains (150 - 160 days). Livability did not vary between strains but was slightly higher than the industry targets reported in the Lohmann management guide, which is 90 - 92% during the laying period. Similarly, Tainika et al. (2024b) found similarity in livability when they compared Lohmann LSL Classic and Lohmann Sandy.

In the current study, egg production was similar between strains but the values were dissimilar to the Lohmann management guide. For instance, in the guide, hen house egg production is 199 and 201.5 eggs at 52 wk of age for Lohmann brown and Lohmann LSL Classic, respectively. In contrast to the present data, Sözcü et al. (2021) observed genetic disparity regarding egg production between the Atak-S and Atabey genotypes. Additionally, Tainika et al. (2024b) identified differences in hen day egg production between Lohmann LSL Classic and Lohmann Sandy. Overall, the variation in the results between the present study and the industry targets or some previous studies might be due to differences in factors such as study region, management level, and feeding.

Perch and nesting area treatments resulted in no differences in live body weight at the end of the study, 52 wk of age. This would be in line with the fact that the challenge of adapting to a new environment is short-term or time-bounded, which could not result in longer-term effects on body weight in hens. This indicates that birds can get accustomed to a specific environment over time (Bari et al., 2020), resulting in a lack of variability in parameters such as live body weight and mortality. Indeed, Engel et al. (2019) did not observe differences in body weight between hens housed in cages with or without a nest box. Also, Barnett et al. (2009) reported no difference in body weight between hens in cages with or without a nest box in early (29-36 weeks of age) and late in life (59-66 weeks of age).

In this study, however, hens reared in cages without a perch being heavier than their counterparts at 50% egg production age is probably linked to the fact presence of a perch permitted increased motor activity in the latter and consequently, more nutrients were required to compensate for energy loss than body weight gain. Moreover, the presence of perches in cages has been shown to compromise welfare by increasing the presence of clinical conditions such as keel bone damage and footpad dermatitis, which could have a

detrimental effect on the performance of laying hens (Tauson and Abrahamsson, 1994; Sherwin et al. 2010; Casey-Trott et al., 2017; Dedousi et al., 2020; DePaoli et al., 2024). Nevertheless, the perches used in this study were metallic perches, which have been shown to increase the severity of damage to the keel bone (Käppeli et al., 2011; Stratman et al. 2015). It could be possible the presence of perches compromised the welfare of the laying hens in this study, which further translated into poor performance however, only a few weeks after the beginning of the study.

In the current study, there was no effect of cage structural items on mortality and egg production traits, which is consistent with some previous studies. For example, Duncan et al. (1992) identified no significant difference in hen day egg production across 12 laying periods between hens housed in cages with perches and without perches. Also, Barnett et al. (2009) did not find variations in hen day egg production between hens in cages with or without perches. In addition, Engel et al. (2019) reported similar hen day egg production between hens housed in cages that had and those that lacked a nest box. It can be argued that a lack of difference in production variables among all the treatments could be attributed to the bird's adaptability to the new environment, resulting in a lack of associated changes in biological systems and functioning related to productive and reproductive traits. Furthermore, the production performance data in this study would also indicate the familiarity of birds with the new circumstances. This could as well be justified by a study involving a different aspect associated with enriched cage perch, where body weight and egg production did not differ among hens from 24 to 40 wks of age reared in cages with either circular steel or mushroom plastic perch designs (Tarim et al., 2024).

In the study, the genetic influence on overall egg weight was higher for Lohmann brown than Lohmann LSL Classic which is in line with Lohmann (2021). Similarly, some authors (Lordelo et al., 2020; Hammershøj et al., 2021; Krawczyk et al., 2023) have reported genetic influence on egg weight. Since the increase in body weight has been associated with the increase in egg weight due to the increase in nutrient intake (Leeson and Summers, 1987), it is speculated that the genetic make-up of the Lohmann brown hens might have provided those birds with the advantage of being able to consume more feed and transfer more nutrients in eggs compared to the Lohmann LSL Classic.

In the present study, the higher overall egg weight

identified in cages without a perch than those with a perch would be consistent with Duncan et al. (1992), who reported similarity in weights of eggs obtained from hens housed in cages with or without a perch. Leeson and Summers (1987) revealed that the increase in hen body weight is associated with the increase in nutrient intake, and consequently, the increase in egg weight. Thus, the data in the present study might indicate that the presence of a perch was linked to increased motor activity of hens, leading to increased energy requirement and so, less consumable nutrients could be deposited in eggs, leading to lower egg weight. Moreover, perch availability in cages has been reported to influence some clinical conditions (keel bone damage) in hens, and hens with keel damage would use most of the Ca in feed to heal these damages, leading to lower egg production. This scenario has been reported to have a negative effect on egg weight (Nasr et al., 2013; Nasr et al., 2012).

The egg-laying time-related effects on overall egg weight are in agreement with some authors. For example, Tůmová et al. (2009) observed heaviest eggs at 6:00 a.m. compared to 10:00 a.m. and 2:00 p.m. Eleroğlu and Taşdemir (2020) found heavier eggs at 10:00 a.m. and noon compared to 3:00 p.m. Eleroğlu (2021) and Krawczyk et al. (2023) determined heavier eggs at 1:00 and 3:00 p.m. than at 7:00 and 10:00 a.m. and noon, respectively. Akyol et al. (2024) identified the heaviest eggs at noon, followed by 9:00 a.m., then 3:30 p.m. Tainika et al. (2024b) reported that more large eggs were collected at 9:00 a.m. and noon than 3:00 p.m. However, more extra-large eggs were obtained at 9:00 a.m. than at noon and 3:00 p.m. Usually, the slight variations in overall egg weight based on the specific time might be linked to the total number of eggs that could be weighed at each time and consequently, the study period.

As would be expected, overall egg weight increased as the flock age increased. Some authors (Alig et al., 2023a, 2023b; Tainika et al., 2024b) have reported this effect.

In this study, the observed effect of nesting area treatment on yolk color score is poorly understood, especially where the intensity of the yolk is majorly associated with what the birds consume (Zurak et al., 2022). Furthermore, we would relate the lower intensity of yolk color of eggs in hens reared in cages without a nesting area to increased stress responsiveness, which is one of the factors affecting the coloration of yolk (Zurak et al., 2022). In addition, although the present

data identified similar stress responses for nesting area treatments, it could be possible that the limitation of the nesting area negatively influenced the affective state of the hens, which might have triggered some physiological and behavioral changes and imbalances, leading to stress responses enough to modulate yolk coloration. However, material treatments did not influence other egg quality traits. Similar to this study, Duncan et al. (1992) identified no difference in egg weight between eggs from hens in cages with or without perches. Alm et al. (2016) reported no differences in the percentage of eggs with shell irregularities (wrinkled top, pimples, spots, stripes, and thin shells) between hens in cages with or without access to a nest box. Engel et al. (2019) observed similar egg weights across age of hens, between 26 and 29 wk of age. In the current study, it can be argued that there were no changes in related physiological responsiveness in hens in different treatments, resulting in lack of adverse effects on egg quality traits. It is also speculated that although the treatments had some significant effect on other parameters (overall egg weight, yolk intensity etc.), hens were able to rapidly acclimatize to their environment, and continue with normal egg production. This reason could account for the lack of the difference in egg quality traits observed.

Generally, literature is scarce on the impact of exclusion and inclusion of enriched cage materials on egg quality variables thus, further studies are required. This would aim to fully understand the optimization of enriched cages to enable management decisions and their faster implementation in countries where conventional cages are still being utilized.

Furthermore, the interaction effects observed in some egg quality traits in the present study might be related to the pattern of changes in physiological responses associated with feeding behavior as birds cope with the cage environmental changes associated with different rearing treatments, especially with the aging of hens.

Several studies identified genotype differences in the ratio of eggs with meat and blood inclusions (Jeffrey, 1945; Campo and Gil, 1998; Hammershøj et al., 2021; Akyol et al., 2024; Tainika et al., 2024c). This contradicts the study by Lordelo et al. (2020) and the present study. In the current study, the impact of perch treatment on meat-blood spots in egg albumen is poorly understood, warranting further studies. However, Campo and Gil (1998) reported that the occurrence of meat and blood spots in eggs involved changes in fear and stress responses in hens. Furthermore, other

suggested factors that can increase meat and blood inclusions in eggs include fright and high perches (Nalbandov and Card, 1944), and cage system compared to deep litter and free-range (Şekeroğlu et al., 2010).

Some authors (Nalbandov and Card, 1944; Jeffrey, 1945; Jensen et al., 1952) reported a significant impact of hen age on meat-blood spots in eggs, which would be in line with the present study. However, the above authors observed a declining trend in meat-blood spots in eggs as the season advanced in eggs. This would contradict the current results where the blood spots in the yolks were significantly the highest at 52 wk of age although a decreasing trend had been identified from 34 to 47 wk of hen age. On the other hand, Hammershøj et al. (2021) and Akyol et al. (2024) did not identify the age effect on ratio of eggs with blood and meat inclusions. Furthermore, Tainika et al. (2024c) observed the age effect on ratio of eggs with meat and blood inclusions only in the albumen than in the yolk.

Age-related changes in egg quality traits were expected as various authors (Şekeroğlu et al., 2014; Samiullah et al., 2017; Yılmaz Dikmen et al., 2017; Yurtseven et al., 2021; Akyol et al., 2024; Baylan et al., 2024; Şekeroğlu et al., 2024; Tainika et al., 2024c) reported such changes, occurring as the flock age increased.

H/L ratio and duration of TI are well-established physiological parameters and are indicators of stress (Gross and Siegel, 1983) and fear (Jones and Faure, 1981) responses in birds, respectively. In this study, the physiological parameters, that is, white blood cell count and H/L ratio, and duration of TI were not affected by genotype, and perch and nesting area treatment. The results of the present study are consistent with some authors who report no effect of exclusion or inclusion of some furniture elements in cages on welfare traits of hens. For example, Barnett et al. (2009) found no differences in white blood cell count and white cell ratio between hens housed in cages with or without perches. Alm et al. (2016) identified similar H/L ratios and duration of TI between birds that were denied and those that had continuous access to a nest box. It is speculated that the degree and severity of changes in physiological and behavioral responses of the hens due to the exclusion of perch or nesting area was not detrimental enough to cause a lasting stress effect on hens. It could also be possible that the hens were able to rapidly acclimatize to their housing environmental.

Furthermore, studies that relied on corticosterone hormone concentrations in hen tissues also did not identify differences in stress responses between hens reared in cages with the presence or absence of some furniture items. For example, cages that had or lacked a perch (Barnett et al., 2009), cages with or without access to a nest box (Engel et al., 2019), and cages with a closed and opened nest box (Alm et al., 2016). Generally, similar to the conclusion of Engel et al. (2019), the exclusion of perch or nesting area may not be able to disrupt the biological functions associated with stress and fear responses in hens. The reason may be that the effects of an environmental stressor can be severe but for a short time (Nicol, 2015), indicating the influence of the sampling time.

In the current study, there was no genetic influence on feather condition scores contrary to what has been reported by several authors (Morrissey et al., 2019; Tok et al., 2022; Tainika et al., 2024a). The variation in the results could be related to factors including the differences in the genotypes that were studied, sampling time, level management, housing system and conditions, and study region. The current study observed better feather condition scores in hens in cages without a nesting area. This is in agreement with Engel et al. (2019) who found worse feather scores in hens that had access to a nest box at 30 and 34 weeks of age. It could be argued that the abrasion with the next box may be responsible for the increased feather damage (as stated by Engel et al., 2019). In contrast, Alm et al. (2016) reported no difference in feather cover between hens housed in cages excluded from the nest box and those with continuous access to a nest box.

In the present study, feather condition scores between hens in cages with or without perch did not differ. Barnett et al. (2009) identified no variation in feather condition scores between hens housed in cages with or with perch early in life of hens (29-36 wk of age). However, the later authors found significantly higher feather scores in birds without perches than with perches in late life (59-66 wk of age). Engel et al. (2019) only found better feather condition scores in hens that lack a nest box than those with access to a nest box at 34 wk of age but similar scores at 26 and 30 weeks of age. It is not clear whether differences in genotypes, age of hens at assessment, study region, etc. would be the source of variation in results of the studies among contradicting studies.

Also, as would be expected and reported by some

authors (Morrissey et al., 2016; Tok et al., 2022; Tainika et al., 2024a), feather loss increased with the aging of hens in the present study. It is well-known that birds are less vulnerable to environmental temperature as they can regulate body temperature (Smith and Oliver, 1971). Kamar and Khalifa (1964) and Kim et al. (2020) reported that the trend in variation in body temperature was connected to similar variations in air temperatures. The present study identified genetic influence on all body surface temperatures except for rectal temperature, which might be argued as a result of both genotypes being developed from breeds with dissimilar adaptability levels to cope with the changing environmental temperatures. Indeed, Kamar and Khalifa (1964) emphasized that body temperature is a breed characteristic. However, the latter authors reported breed differences in cloacal temperature, which was associated with differences in activities and body size of birds. Furthermore, some authors observed a genetic influence on comb and rectal temperatures (Tainika et al., 2024a) and only the rectal temperature (Akyol et al., 2024).

In the present study, the lack of differences in some body temperatures in hens might be attributed to the similar variation in air temperature in cage treatments. However, it is not certain whether the influence of perch and nesting area treatments on some body temperatures in hens might be associated with the variability in the level of activity in the treatments and body size of hens as determined by Kamar and Khalifa (1964). Therefore, the effect of cage furniture items on body temperature is not fully understood, warranting further studies. Collectively, the level of motor activity because of the presence of some furniture items in the housing environment might modulate some body temperatures of hens. For instance, the higher footpad surface temperature observed in hens in cages with perch could be linked to increased perching behavior.

The interaction effects observed in some body region temperatures in the present study might be attributed to the pattern of change in thermal regulation because of bird's adaptability to the cage environmental changes associated with different rearing treatments, especially with the aging of hens.

Generally, the welfare of laying hens can play a major role in performance and egg quality traits and so, identifying the most important furniture features, with the greatest potential to improve the welfare of commercial laying hens in cages is important in en-

suring sustainable production. Consequently, this has a greater potential to increase the economic returns of farmers. It is worth noting that there is a ban on conventional cages for the rearing of hens in many developed countries however; without a doubt, in many parts of the world, conventional cage systems are still the most common production system for laying hens. This is due to the fact that they ensure increased egg yield and quality, which are among the critical factors influencing the profitability of commercial farms. Meanwhile, the data in the current study does not offer any evidence that the inclusion of furniture items enhances the welfare of hens and production performance and egg quality traits that farmers consider significant in terms of revenues. Nevertheless, the concept of this study has not yet been exploited enough and this research could serve as one of the efficient preliminary studies for future comparative studies between the available and future furniture items to ensure that they come with benefits beyond permitting the associated behavioral repertoire only. This is because producers in the regions utilizing conventional cages still need to understand the benefits of enriched cages from the production performance perspective and this is what should be considered while developing environmental features that come with additional production costs.

## CONCLUSIONS

The present results indicate that excluding a perch in enriched cages increased live body weight at the age at 50% egg production and meat spots in albumen. The inclusion of a perch in cages led to increased blood spots in albumen. The lack of a nest box in enriched cages resulted in an earlier age at 50% egg production and better feather condition scores than the inclusion of a nesting area in cages. Overall, the study did not find differences in production, egg quality traits, and welfare variables due to the strain and exclusion or inclusion of a perch or nesting area in enriched cages. However, age-related changes in feather cover score, egg quality traits, meat and blood inclusions in the yolk, and body region temperatures were demonstrated. Generally, the influence of strain and structural elements of enriched cages on the performance outcomes of hens warrants further studies to be refined.

## CONFLICT OF INTEREST

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

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