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# The effect of housing environment (deep litter with or without access to different plant species outdoor) on welfare and behavior across two strains of laying hens

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**Abstract:** The impact of the housing environment and hen strain on the welfare and behavior of laying hens was the focus of this study. Lohmann LSL Classic (W) and Lohmann Sandy (S) were assessed under deep litter without outdoor access (DL), deep litter with access to outdoor pens covered with either *Mentha piperita* (MP), *Petroselinum crispum* (PC) or *Medicago sativa* (MS). Duration of tonic immobility, feather condition, footpad dermatitis, and temperatures (comb, breast region, footpad surface, and rectal) were determined at 31, 42, and 52 weeks of age. Hen's behaviors were observed at 32, 42, and 52 weeks of age. Blood parameters were assessed at 52 weeks of hen age. Duration of tonic immobility was similar between hen strains and across ages of hens ($p > 0.05$) but nearly reached significant levels due to the housing environment ($p = 0.070$). There was a significant effect of age ($p < 0.001$), housing environment ($p < 0.001$), and hen strain ($p < 0.05$) on total feather score. Age-related effects were observed for all the body region temperatures ($p < 0.001$), and only the comb and rectal temperatures differed between hen strains ($p < 0.001$; $p < 0.05$). The housing environment did not affect the body surface temperatures ($p > 0.05$). Blood parameters did not differ between hen strains and housing environments ($p > 0.05$). There was a significant effect of age, housing environment, and hen strain on the proportion of hens expressing some behaviors ($p < 0.001$; $p < 0.05$). Time of the day influenced the proportion of birds expressing wing flapping and dust bathing behaviors ($p < 0.05$). It was concluded that feather condition varies with the housing environment and hen strain, duration of tonic immobility may differ across housing environments, but other welfare traits remain to be refined. Also, housing environment but not strain modulates the expression of most behaviors of hens.

**Key words:** Aromatic plants, behavior, housing environment, laying hen, welfare

## 1. Introduction

It is well-established that the publication entitled "Animal Machines" by Ruth Harrison in 1964 and the Brambell report in 1965 led to increased ethical concerns about the housing systems for laying hens [1,2]. This resulted in welfare developments including the ban on conventional cages in the European Union (EU) in 2012 [3] and the widespread use of enriched cage systems. However, recently, consumers in several developed countries have continuously argued that the enriched cage system is also not animal-friendly compared to cage-free systems. While a ban is in place on the housing of laying hens in enriched cage systems in countries such as Sweden, Switzerland, and Germany, the system is expected to be banned in the EU over time. Thus, housing laying hens in cage-free systems is expected to become popular [4].

Hen welfare remains a subject of interest in the poultry industry. Some of the parameters for assessing hen welfare include fear responses, feather coverage, footpad health, stress response, and behavior [5,6]. Fearfulness in birds is commonly assessed by the duration of tonic immobility (TI) [7]. Fear has a genetic basis and varies between strains of laying hens [8–11]. Furthermore, fear is also influenced by the housing system the hens are kept in as well as the age of birds [8,12].

Blood parameters, especially the heterophil-to-lymphocyte (H/L) ratio, are commonly employed indicators of stress response in birds [13]. Blood parameters vary between strains of laying hens [8,9,14], housing environment [8,15,16], and the ages of the hens [17].

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Feather coverage of laying hens has a genetic basis and differs between strains of laying hens [14,18–20]. Feather coverage is also influenced by the housing environment [8,19] and deteriorates with the increase in the flock age [14,18,21,22]. Footpad health usually worsens to a varying degree based on the strain of laying hens and the condition of the housing system [19,20].

The body temperature of hens is also a well-known indicator of animal welfare, which is mainly influenced by the characteristics of the housing system, especially in terms of environmental temperature changes [23–25], and can vary based on the genetics of laying hens [23].

Meanwhile, behavioral observation can also be an assessment tool for hen welfare. Freedom to exhibit behaviors is influenced by factors including the housing environment, and the features of the housing environment [26,27]. For example, while the engagement in natural behaviors is reduced due to the lack of opportunities or resources [28], the increased expression of natural behaviors especially due to increased opportunities has been associated with positive hen welfare [29]. Expression of behaviors is also influenced by features in the specific location and age of laying hens [29].

It is worth noting that access to different plant species of nutritional relevance such as aromatic plants in outdoor areas might modulate the behavior and welfare of laying hens differently when they are eaten and ranged on. However, there is a void of information about the utilization of aromatic plant species in outdoor areas for the optimization of free-range laying hens. Furthermore, in the 21st century, the laying hen strains selected for cage-free systems could also differ in how they adapt to outdoor areas vegetated with different plant species. It is argued that to produce laying hens in a welfare, healthy, and environmentally friendly way, various aspects are of significance.

Therefore, this study aimed to evaluate the effect of housing environment (deep litter without and deep litter with access to different outdoor plant species: *Mentha piperita*, *Petroselinum crispum,* and *Medicago sativa*) on the welfare and behavior of two laying hen strains (Lohmann LSL Classic and Lohmann Sandy).

## 2. Materials and methods

The study was approved by the animal welfare ethical committee of Niğde Ömer Halisdemir University (approval number: 2021/04).

### 2.1. Establishment of plant species

Within each outdoor pen, soil preparation involving plowing, removal of stones, and leveling without herbicide treatment was applied. Subsequently, the pens were randomly allocated to plant treatments and their corresponding replicates, initiating the planting process. Each outdoor pen measured 9.41 m × 1.94 m (total area = 18.25 $m^2$).

*Mentha piperita* was grown through vegetative propagation using suckers or sprigs. These were initially cultivated in pots within the greenhouse. Once established, the pots comprising fully developed shoot systems were transported to the designated planting area. The suckers were then planted in rows and columns, ensuring an approximate spacing of 10 cm between each plant sucker to achieve a dense plant cover (200 suckers of *Mentha piperita* per square meter).

*Petroselinum crispum* and *Medicago sativa* were established using seeds obtained from a certified seed dealer in the province. The recommended seed rates were 183 g per pen (equivalent to 10 g per square meter) for *Petroselinum crispum* and 275 g per pen (equivalent to 15 g per square meter) for *Medicago sativa*. Before sowing, the seeds were combined with 1 kg of artificial fertilizer and then broadcasted across the planting area. Moreover, following the guidance of an experienced professional in forage plants, sacks were used to cover the sown land in pens designated for *Petroselinum crispum* to prevent the seeds from drying out, as they are highly sensitive to direct sunlight.

The sowing process took place in the late evening over two consecutive days. Sprinkler irrigation was ensured twice (morning and evening) every day up to when the seeds germinated. This was followed by flood irrigation once a day until the plants completely covered the pens. Additionally, weeds were regularly pulled out as soon as they were identified.

The plant species were regularly mowed and maintained to a uniform height of 20 cm before granting the birds access to the outdoor pens beginning from 12 weeks of age. It is important to mention that throughout the study, watering was ensured daily in the evening after closing the pop holes at 3:30 p.m. to maintain the forage quality, except for rainy days and throughout the entire winter season. Additionally, weeds were regularly removed from the range areas to avoid their detrimental impacts.

During the period the birds were permitted access to the range, plant management practices including rotational foraging, fertilizer application, and mowing were not applied.

As a note, from August to September, the outdoor pens were densely covered with plants, while in October, approximately 60% of plant coverage was estimated. By November, the plant coverage had decreased to approximately 30% probably due to the hens' activities causing their depletion. In December, there was almost no plant coverage, and from January to February, there was absolutely no coverage except for the standing stem parts without leaves. This lack of coverage during winter can be attributed entirely to the influence of winter weather. The regrowth of plants started to occur in March.

**2.2. Birds, housing, and management**

The study was conducted from June 2022 to May 2023 at Ayhan Şahenk Agricultural Application and Research Center of Niğde Ömer Halisdemir University (37°58′ North 34°40′45 East, elevation; 1299 m) in Niğde province, Türkiye. All the housing environments were sited in the same barn.

Beak-trimmed laying chicks from two strains (total = 300), Lohmann LSL Classic (W, n = 150) and Lohmann Sandy (S, n = 150) were obtained from the same breeder company. The chicks were three weeks old when brought to the study barn. The selection of these strains for the study was in accordance with the degree of popularity at the commercial level. Briefly, the W hybrid line is well-known for its efficient production of white eggs and is considered a universal hybrid. On the other hand, the S hybrid is not yet widespread in the poultry industry. However, it is a white-feathered hybrid line that produces cream-colored eggs and is characterized by a good feed conversion ratio and robustness[1].

At the experimental unit, the chicks were first reared in two litter floor pens based on their hybrid line for one week to acclimatize to the new environment. At 4 weeks of age, the birds were individually weighed, and a total of 10 birds per hybrid line were randomly placed in one of the 26 experimental replicate pens (13 flocks of each hybrid line). The experimental pens had been predetermined during the establishment of outdoor plant species. Thus, a total of 260 birds were used in this study.

The chicks were first kept in the same rearing conditions which consisted of a concrete floor with a layer of wheat straw litter (8 cm), and in fixed indoor pens of wire mesh walls measuring 2.79 $m^2$. During the study, new litter was added whenever it decreased, and replacement of litter was carried out every time caking was detected. Indoors, birds had *ad libitum* access to feed (one hanging feeder, 41 cm in diameter) and water (one round bell drinker, 30 cm in diameter). Later at 19 weeks of age, a 3 × 3 (tier and cell) metallic laying nest box measuring 98 cm × 37 cm × 138 cm was added in. Indoor stocking density was 3.58 birds/$m^2$ and was not determined in relation to the area occupied by the above items.

During the early weeks of chick brooding, heat was provided by electric heaters with internal temperatures maintained at approximately 23 °C to facilitate the adaptation to the ambient temperatures in outdoor areas. Subsequently, from 8 weeks of age until the end of the study, the temperature was allowed to fluctuate freely. Automatic fans were turned on to cool the environment in case of extreme elevation of internal temperature, and throughout the study, there were no observed signs of heat stress in the birds. Also, in cases of extremely low temperatures, the electric heaters were turned on. The hens were fed standard concentrate layer feed (purchased from a private company) as shown in Table 1.

The light program (light, L to dark, D) of 13L:11D was offered to the birds from three weeks of age followed by a

**Table 1.** Ingredients and composition of the commercial feed used at various intervals during the study.

| **Nutrient composition** | **Type of feed (age of hens)** | | | | |
|---|---|---|---|---|---|
| | Layer grower (3–8 weeks) | Layer developer (8–18 weeks) | Peak lay (18–23 weeks) | Layer 1. Phase (23–33 weeks) | Layer 2. Phase (34 weeks until the end of the study) |
| Crude protein, % | 20.7 | 16 | 17.5 | 17 | 15.61 |
| Crude cellulose, % | 3.9 | 4.3 | 3.6 | 4.5 | 4.8 |
| Crude ash, % | 5.2 | 5.5 | 13.6 | 13.7 | 12.2 |
| Crude fat, % | 3.6 | 2.2 | 4.4 | 4.9 | 3.83 |
| Calcium, % | 0.2 | 1.2 | 3.9 | 3.9 | 3.83 |
| Phosphorous, % | 0.4 | 0.4 | 0.4 | 0.4 | 0.42 |
| Sodium, % | 3.9 | 0.2 | 0.1 | 0.1 | 0.16 |
| Lysine, % | 1 | 0.8 | 0.8 | 0.8 | 0.76 |
| Methionine, % | 0.5 | 0.4 | 0.4 | 0.4 | 0.37 |
| Metabolic energy, kcal/kg | 2700 | 2700 | 2700 | 2700 | 2700 |

Ingredients: *Maize, **Soya bean meal, wheat, calcium carbonate, sunflower seed meal, *Dried distillers grains (DDGS), soya oil, dicalcium phosphate, sodium chloride, sodium bicarbonate. *: Produced from genetically modified maize, **: Produced from genetically modified soya
Vitamin A 12,000 IU; Vitamin D3 2400 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

[1]Lohmann (2022). Management guide, Alternative systems [online]. Website LB_eMG_Alternative-Haltung_Printversion_EN_06.21_V01-21_high.pdf (lohmann-breeders.com) [accessed 22 04 2023].

step-down program of Lohmann management guide until 17 weeks of age. Afterward, the photoperiod was adjusted as follows. At week 18: 10L:14D, 19 weeks: 10.30L:13.30D, 20 weeks: 11.15L:12.45D, 21 weeks: 12L:12D, 22 weeks: 12.45L:11.15D, 23 weeks: 13.30L:10.30D and at 24 weeks: 14L:10D. Subsequently, the lighting period was increased weekly by 30 min until it reached 16L:8D at 27 weeks of age, which was maintained up to 52 weeks of age. The lighting schedule was automated, and the barn was illuminated by white light sourced from warm LED bulbs of 14 watts/2700 K. The light bulbs were cleaned with a cloth on a regular basis to avoid dust accumulation.

Before introducing the birds to the poultry experimental facility, a vaccination program in line with the guidelines of the breeder firm had been implemented until 3 weeks of hens' age. Afterward, the birds were vaccinated against Infectious bronchitis and Newcastle (Ma5+Clone30 in drinking water) at 11 weeks of age and fowl pox (VAIOL-VAC via wing web) at 23 weeks of age. Furthermore, a mixture of vitamins and amino acids via drinking water was offered to the birds after the vaccination process. In the study, five mortalities were recorded.

Besides the indoor characteristics as explained above, the pens (n = 16) for the deep litter environment (DL) were in the center of the poultry house. The DL environment was separated from the indoor pens of the deep litter with access to various plants by a corridor of at least two m on both sides of the poultry house. In the study, birds were randomly distributed to only eight DL pens: four replicate pens per hybrid line (10 birds/pen), and the birds were reared completely indoors throughout the study. The remaining eight pens were not utilized during the study but, two of these pens were used for rearing the remaining 40 birds that were not part of the study until they were sold.

For the deep litter with access to different plant species in outdoor areas, each indoor pen had a pop hole (50 cm high × 50 cm wide) in the center so that the birds could access the outdoor pens (total area = 18.25 $m^2$ per pen). Each outdoor pen was covered with one of three plant *species: Mentha piperita* (MP), *Petroselinum crispum* (PC), or *Medicago sativa* (MS). There were three replicate pens per treatment each consisting of 10 birds, with an outdoor stocking density of 0.55 bird/$m^2$ (outdoor area of 1.825 $m^2$/bird). The pop holes were opened daily and continuously between 8:30 a.m. and 3:30 p.m. from 12 weeks of hens' age until the end of the study (52 weeks of age). Outdoor pens were fenced and separated by the wire mesh wall, avoiding the movement of birds from one pen to another. Due to the open top of these pens, some particular birds (n = 8) could fly to the neighboring pens. This was prevented by trimming the flight feathers of one wing of the birds before they were placed back in their original pens.

It is important to note that hens were not granted range accessibility in the first three weeks of February because of extreme cold and snowfall. It was thought that this weather would negatively influence the well-being of hens.

In addition, the vegetation in the two pens that were not used was regularly mowed to avoid shade that could result in a nonuniform environment across the pens. Additionally, the barn lacked a veranda, and aside from the experimental plant species, other opportunities including trees or shelters were not available in the outdoor pens.

It should be emphasized that the study was performed between June 2022 to May 2023, indicating wider fluctuations in weather. When the birds housed in the outdoor-based environments were allowed access to the range areas, outdoor temperatures ranged from 23–35 °C in August, from 8–34 °C between September to November, from 3–19 °C between December to February, and from 9–25 °C between March to May. Indoor temperatures ranged from 20.9–31 °C degrees in August, from 5.6–30.3 °C between September to November, from 1.1–15.1 °C between December to February, and from 4–20.1 °C between March to May

**2.3. Duration of tonic immobility (TI)**

At 31, 42, and 52 weeks of age, two hens from each replicate pen were tested for TI duration. The TI test was performed by a single assessor, an experienced doctoral researcher in poultry welfare. At 31 weeks, one hen at a time was randomly caught from the respective pen and carried to a separate room within the same poultry house for testing TI. Before each hen was returned to its respective pen, both legs of the first hen and only the left leg of the second hen were marked with aerosol spray paint black. This is because the same birds were to be tested at 42 and 52 weeks of age. Within the testing room, the hen was immediately placed on its back on a U-shaped wooden cradle with the head hanging freely. TI was induced by the assessor placing one hand on the bird's breast region and the other hand on the head. After 10 seconds (s), the assessor removed his hands, and the stop clock was started. Also, the assessor was positioned at least 1 m away, and his eyes were in direct view of the hen to induce her fear response. The time in seconds taken by the hen to right (returning to normal position) was recorded as the duration of TI. When the bird returned to normal position less than 10 s after the assessor removed his hands, it was considered an unsuccessful attempt at TI induction (no TI). Therefore, the assessor immediately repeated the induction procedure but not more than three attempts. In case the TI could not be induced after three attempts, the hen was deemed unsusceptible, and her duration of TI was scored as 0 s. On the other hand, when the hen did not return to its normal position after 10 min, a maximum score of 600 s was recorded as the duration of TI [7].

**2.4.Feather condition (FC) and footpad dermatitis (FPD)**
FC and FPD were conducted by a single experienced researcher in poultry welfare assessment at weeks 31, 42, and 52 of hens' age. The same birds that had been tested for TI were assessed for FC and FPD (two hens per replicate pen). FC was assessed by feather scoring which was based on the degree of feather loss and a 4-point scoring scale was used: 1- complete feather loss, 2- 50% feather loss, 3- 25% feather loss, and 4- full feather coverage. The six regions: the head, neck, breast, back, wings, and tail of each hen were scored separately. Thereafter, the total feather score for each bird was calculated. While a total of 6 points from the six body parts of chickens indicated that they lost all their feathers, a total of 24 points showed that they had all their feathers maintained [22]. FPD was assessed using a 3-point scoring scale (0–2) according to the welfare quality protocol [5].

**2.5. Body region surface and rectal temperatures**
The temperatures of two hens per replicate pen were measured at weeks 31, 42, and 52 of age after they were assessed for TI. Rectal temperature (°C) was measured by inserting the digital thermometer into the cloaca (about 3 cm deep). The digital thermometer (MEDIX KD-106, China) was kept in the cloaca of the hens until the temperature increase stabilized. The breast region, comb, and footpad surface temperatures (°C) of the hens were determined by the infrared thermometer (LOYKA DARK II, China).

**2.6. Behavioral assessment: video recording and data collection**
Handheld cameras (Sony HDR-CX190E, China) were used to capture the birds in the replicate pen areas for five min once at 32, 42, and 52 weeks of age in the morning (8:00 a.m.– 12:30 noon) and in the afternoon (1:00–4:00 p.m.). All the replicate pen areas were video recorded by three persons without intervals within the above specified time ranges. The three persons were all trained by a single researcher on how to use or adjust the cameras and correctly capture all the birds inside the replicate pen areas. The persons continuously approached each pen slowly and stood at least one to two meters away from the pen (outside the pen) under observation to reduce the risk of disturbing the hens. For each observation time, all the 18 outdoor pens of the different plant groups were first recorded by two persons: person one started from pens 1–9 (front side of the barn) and person two from pens 18–26 (back side of the barn). Person three started from the inside pens of the deep litter environment (pens 10–17). Afterward, persons one and two recorded the indoor pens of the different plant groups following the same order as above. For the free-range system, all the pens were captured regardless of the number of hens in outdoor and indoor areas during the observation time. On the other hand, all the video recordings were decoded by a single experienced doctoral researcher (observer) in poultry behavior to ensure the identification of correct behaviors. One behavior at a time, the birds expressing that specific behavior were counted across a five-min video recording for each replicate pen. The behaviors were expressed as a percentage of the total number of birds per replicate pen. The ethogram that was followed during the determination of hens' activities was a modification of Campbell et al. [29], as shown in Table 2.

**Table 2.** Ethogram of behaviors that were scored, modified from Campbell et al. [29].

| **Behavior** | **Description** |
|---|---|
| Wing flapping | Outstretching and rapid flapping of both wings while the hen is on the ground |
| Stretching | While standing or lying, the hen stretches one leg and one wing on the same side |
| Preening | With the beak, the hen is aligning or pulling off dirt from her feathers |
| Drinking | Hen standing in front of the drinker and drinking water |
| Feeding | Hen standing in front of the feeder and pecking the feed |
| Pecking other hens | Hen gently or aggressively touches another hen with her beak, also involving feather pecking and pulling |
| Dust bathing | While lying on the ground, the hen is kicking loose particles on to her feathers and throwing them over her body using the wings and full body movement |
| Foraging | Hen is scratching her feet backwards in the dirt followed by ground pecking |
| Walking | Hen is moving slowly between two points |
| Standing | Hen is on her feet and remains in one position (stationary) without pecking |
| Pecking objects (e.g., ground, and plants) | Hen touches objects other than feed in feeders and other hens with her beak |

**2.7. Blood sample collection and determination of blood parameters**

At week 52 of age, 1 mL of blood sample was collected with 2cc sterile syringes from the wing vein of all the birds after they had been tested for TI: two birds from each replicate pen. A single drop of blood was put on the base or smear slide and thereafter, a spreader slide was used to run the dropped blood to ensure that a thin blood smear on the base slide was drawn. The smear slides were then stained with the May-Grunwald and Giemsa stains. Afterward, each hen's slide was examined with the inversion lens microscope, and a total of 100 leukocytes (heterophil, eosinophils, basophils, lymphocytes, and monocytes) were counted to determine the leukocyte formula. The percentage of the heterophil relative to lymphocyte cells was calculated to determine the H/L ratio [13].

**2.8. Statistical analysis**

The assumption of normality of the data was examined by Kolmogorov Smirnov, Skewness, and Kurtosis tests. Parametric methods (analysis of variance and multiple comparison tests) were used in the analysis of variables that provided the assumptions, and nonparametric methods (permutation tests) were used in those that did not provide them. The effects generally examined in the study and the interactions of these effects were given in the following model.

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

In the model; $\mu$: population mean, $\alpha_i$: i. age effect, $\beta_j$:j. housing environment effect, $\gamma_k$: k. strain effect, $\delta_l$: l. time of day effect (morning, afternoon), $\alpha\beta_{ij} + \alpha\gamma_{ik} + \cdots + \alpha\beta\gamma\delta_{ijkl}$: interaction effect, $\varepsilon_{ijklm}$ : random error $(\varepsilon \sim N(\mu, \sigma^2))$.

The analyses in the study were carried out with three different models according to the fact that the variables examined were affected by external factors. Duration of tonic immobility and analysis of some body characteristics (1), blood parameters (2), and behavior analyses (3). Since it was seen that the variables used in the analysis of tonic immobility and some body characteristics (body region temperatures and total feather score) were normally distributed, the effect of age (,α-i.), the effect of the housing environment (,β-j.), the effect of strain (,γ-k.) and the interactions of these effects (,αβ-ij.+,αγ-ik.+,βγ-jk.+,αβγ-ijk.) were investigated by analysis of variance. In cases where the interaction effects were significant, each interaction group was analyzed by one-way analysis of variance, and the differences between the groups were determined by Duncan's multiple comparison test.

For the analysis of the blood cell count data, the assumption of normality was examined, and it was determined that normal distribution was provided for the lymphocyte, monocyte, and heterophile variables (Group 1), while the normal distribution was not provided for the eosinophil, basophil, and H/L ratio variables (Group 2). For this reason, Two Way Anova Analysis was applied to determine the main (housing environment $(\beta_j)$ and strain $(\gamma_k)$) and interaction effects $(\alpha\beta_{ij})$ in the 1st group. In the analysis of the variables in the second group, the permutation test was applied, and the main effects (housing environment $(\beta_j)$ and strain $(\gamma_k)$ and the effects of the interactions $(\alpha\beta_{ij})$ were examined with the Two-way PERMANOVA method. In Two-way PERMANOVA, Euclidean distance and 10,000 permutation numbers are used as distance criteria. When the data obtained were examined, no difference was determined in general and therefore multiple comparison methods were not used.

In the analysis of behavior data expressed as a percentage, it was determined that the variables examined were not normally distributed. For this reason, angle transformation was applied to the data, and it was determined that the data met the normality assumption. By providing the assumptions, all the main effects and all interactions of these effects in the model given above were subjected to analysis of variance. Duncan's multiple comparison test was used to determine the differences between groups. Data obtained by angle transformation were used for intergroup comparisons. Actual averages were given as descriptive statistical values.

SPSS 29 statistical package program was used for the analysis of parametric methods (analysis of variance, Duncan's multiple comparison test) and Past3 statistical package programs were used for the analysis of nonparametric methods (permutation test).

## 3. Results

The age of hens, laying hen strain, housing environment, and their interactions did not significantly affect the TI duration ($p > 0.05$; Table 3). However, the effect of the housing environment nearly reached significant levels ($p = 0.070$).

The results of the feather condition are shown in Table 3. The feather condition significantly deteriorated during the study period with the aging of hens ($p < 0.001$). The total feather score was highest for 31 weeks of age (i.e. better feather condition) and lowest for 52 weeks of age (i.e. worst feather condition). Total feather score differed among the housing environments ($p < 0.001$); lowest in DL hens compared to the other groups whose scores were statistically similar. There was a laying hen strain difference with a higher total feather score in the W than in the S strains ($p < 0.05$). The housing environment * age interaction significantly affected the total feather score ($p < 0.001$; Table 4). DL hens had the lowest total feather scores at both 42 and 52 weeks of age compared to the other

**Table 3.** Body region temperatures (°C), duration of tonic immobility (seconds), and total feather score due to housing environment and laying hen strain when the hens were tested at 31, 42, and 52 weeks of age.

| Age of hens (A) | Comb surface temp | Breast region surface temp | Footpad surface temp | Rectal temp | TI-duration | Total feather score |
|---|---|---|---|---|---|---|
| 31 | 24.06[b] | 35.27[a] | 20.13[b] | 41.39[a] | 258.58 | 24.00[a] |
| 42 | 21.28[c] | 30.77[c] | 14.88[c] | 41.23[b] | 253.37 | 23.25[b] |
| 52 | 29.23[a] | 33.66[b] | 22.61[a] | 41.36[a] | 230.19 | 22.94[b] |
| **Housing environment (HE)** | | | | | | |
| DL | 25.34 | 33.52 | 19.26 | 41.34 | 295.90 | 22.73[b] |
| PC | 24.73 | 33.08 | 19.26 | 41.32 | 208.72 | 23.67[a] |
| MP | 25.73 | 33.35 | 19.87 | 41.29 | 229.89 | 23.80[a] |
| MS | 23.47 | 32.88 | 18.42 | 41.36 | 238.83 | 23.61[a] |
| **Hen strain (HS)** | | | | | | |
| S | 23.59 | 33.27 | 19.73 | 41.37 | 237.15 | 23.22 |
| W | 26.12 | 33.20 | 18.68 | 41.28 | 257.60 | 23.58 |
| SEM | 0.45 | 0.30 | 0.43 | 0.03 | 12.61 | 0.09 |
| **P values** | | | | | | |
| A | <0.001 | <0.001 | <0.001 | <0.009 | 0.706 | <0.001 |
| HE | 0.140 | 0.804 | 0.525 | 0.784 | 0.070 | <0.001 |
| HS | <0.001 | 0.942 | 0.092 | <0.033 | 0.325 | <0.021 |
| A*HE | 0.784 | <0.010 | <0.038 | <0.006 | 0.775 | <0.001 |
| A*HS | 0.340 | 0.788 | 0.124 | 0.941 | 0.952 | 0.146 |
| HE*HS | 0.544 | 0.186 | 0.248 | <0.001 | 0.088 | 0.101 |
| A*HE*HS | 0.168 | 0.342 | 0.304 | 0.103 | 0.794 | 0.690 |

Abbreviations: DL: Deep litter, PC: *Petroselinum crispum,* MP: *Mentha piperita*, MS: *Medicago sativa*, S: Lohmann Sandy, W: Lohmann LSL Classic, SEM: Standard error of means, *: interactions between different factors, TI: Tonic immobility
Means within the same column with different letter superscript significantly differ ($p < 0.05$)

groups. There was no age * hen strain, hen strain * housing environment, and age * hen strain * housing environment interaction effects on feather condition ($p > 0.05$).

During the study period, footpad dermatitis was absent at the ages when the birds were tested in both laying hen strains.

The results of measured temperatures are presented in Table 3. The comb surface, breast region surface, footpad surface, and rectal temperatures differed among the ages of hens ($p < 0.001$). All these temperatures were lowest at 42 weeks of age compared to other weeks. While at 31 weeks of age, the comb surface and footpad surface temperatures were lower than at 52 weeks of age, the reverse was true for breast region surface temperature. The rectal temperature was statistically similar at both 31 and 52 weeks of age. The laying hen strain only significantly influenced the comb and rectal temperature; higher and lower, respectively in the W compared to the S strain ($p < 0.001$; $p < 0.05$). All the temperatures were similar across the housing environments. The age of hens * housing environment interaction effect was observed for breast region surface, footpad surface, and rectal temperatures ($p < 0.001$; Table 4). Both the breast region surface and rectal temperatures were significantly highest in MS hens at 31 weeks of age. Additionally, the breast region surface and rectal temperatures were significantly lowest for MS hens and both MS and MP hens, respectively at 42 weeks of age. The hen strain * housing environment interaction affected rectal temperature; S hens and W hens in the PC environment had the highest and lowest rectal temperature, respectively than other groups ($p < 0.001$; Table 5). There was no age * hen strain, and age * hen strain * housing environment interaction effects on any of the measured body region temperatures ($p > 0.05$).

The age of hens, laying hen strain, housing environment, and their interactions did not significantly influence the measured blood parameters ($p > 0.05$; Table 6).

**Table 4.** Body region temperatures (°C) and total feather score due to the interaction between the age and housing environment when the birds were assessed at 31, 42, and 52 weeks of age.

| A | HE | Breast region surface temp | Footpad surface temp | Rectal temp | Total feather score |
|---|---|---|---|---|---|
| 31 | DL | 34.14$^{a–d}$ | 19.72$^{b–d}$ | 41.47$^{a–c}$ | 24.00a |
| | PC | 35.73$^{ab}$ | 18.81$^{c–e}$ | 41.29$^{bc}$ | 24.00a |
| | MP | 34.92$^{a–c}$ | 20.79$^{a–d}$ | 41.21$^{bc}$ | 24.00a |
| | MS | 36.65$^{a}$ | 21.32$^{a-c}$ | 41.58$^{a}$ | 24.00a |
| 42 | DL | 31.01$^{ef}$ | 14.44$^{fg}$ | 41.28$^{bc}$ | 22.44b |
| | PC | 31.27$^{ef}$ | 17.26$^{d–f}$ | 41.24$^{bc}$ | 23.42a |
| | MP | 31.78$^{de}$ | 16.08$^{ef}$ | 41.19$^{c}$ | 23.92a |
| | MS | 28.92$^{f}$ | 11.87$^{g}$ | 41.20$^{c}$ | 23.50a |
| 52 | DL | 35.40$^{ab}$ | 23.61$^{a}$ | 41.26$^{bc}$ | 21.75b |
| | PC | 32.23$^{c–e}$ | 21.71$^{a–c}$ | 41.42$^{a-c}$ | 23.58a |
| | MP | 33.36$^{b–e}$ | 22.73$^{ab}$ | 41.48$^{ab}$ | 23.50a |
| | MS | 33.08$^{b–e}$ | 22.08$^{a–c}$ | 41.29$^{bc}$ | 23.33a |
| SEM | | 0.30 | 0.43 | 0.03 | 0.09 |
| P values | | <0.010 | <0.038 | <0.006 | <0.001 |

Abbreviations: A: Age of hens when tested, HE: Housing environment, DL: Deep litter, PC: *Petroselinum crispum,* MP: *Mentha piperita*, MS: *Medicago sativa*, S: Lohmann Sandy, W: Lohmann LSL Classic, SEM: Standard error of means, temp: Temperature
Means within the same column with different letter superscript significantly differ ($p < 0.05$)

**Table 5.** Rectal temperature due to the interaction between the housing environment and laying hen strain.

| Housing environment | Hen strain | Rectal temp |
|---|---|---|
| DL | S | 41.32$^{b}$ |
| | W | 41.36$^{ab}$ |
| PC | S | 41.55$^{a}$ |
| | W | 41.08$^{c}$ |
| MP | S | 41.22$^{bc}$ |
| | W | 41.37$^{ab}$ |
| MS | S | 41.41$^{ab}$ |
| | W | 41.31$^{b}$ |
| SEM | | 0.03 |
| P value | | <0.001 |

Abbreviations: DL: Deep litter, PC: *Petroselinum crispum,* MP: *Mentha piperita*, MS: *Medicago sativa*, S: Lohmann Sandy, W: Lohmann LSL Classic, SEM: Standard error of means, temp: Temperature
Means within the same column with different letter superscript significantly differ ($p < 0.05$)

**Table 6.** Blood parameters (%) of hens due to housing environment and laying hen strain when tested at 52 weeks of age.

| **Group 1** | | | |
|---|---|---|---|
| **Housing environment (HE)** | **Lymphocyte** | **Monocyte** | **Heterophil** |
| DL | 51.06 | 20.06 | 26.44 |
| PC | 50.42 | 23.25 | 23.67 |
| MP | 48.08 | 22.08 | 27.33 |
| MS | 47.00 | 21.75 | 28.42 |
| **Hen strain (HS)** | | | |
| S | 49.00 | 21.19 | 27.73 |
| W | 49.58 | 22.12 | 25.19 |
| SEM | 1.33 | 0.81 | 1.75 |
| **P valves** | | | |
| HE | 0.682 | 0.581 | 0.837 |
| HS | 0.877 | 0.524 | 0.515 |
| HE*HS | 0.378 | 0.846 | 0.745 |
| **Group 2** | | | |
| **Housing environment (HE)** | **Eosinophil** | **Basophil** | **H/L** |
| DL | 2.38 | 0.06 | 0.59 |
| PC | 2.50 | 0.17 | 0.58 |
| MP | 2.42 | 0.08 | 0.62 |
| MS | 2.67 | 0.17 | 0.66 |
| **Hen strain (HS)** | | | |
| S | 2.04 | 0.04 | 0.65 |
| W | 2.92 | 0.19 | 0.57 |
| IQR (Min-Max) | 3.00 (0.00–9.00) | 0.00 (0.00–2.00) | 0.46 (0.11–1.93) |
| **P values** | | | |
| HE | 0.988 | 0.886 | 0.978 |
| HS | 0.178 | 0.165 | 0.494 |
| HE*HS | 0.104 | 0.473 | 0.728 |

Abbreviations: DL: Deep litter, PC: *Petroselinum crispum,* MP: *Mentha piperita*, MS: *Medicago sativa*, S: Lohmann Sandy, W: Lohmann LSL Classic, SEM: Standard error of means, IQR: Interquartile Range, Min-Max: Minimum-maximum, *: Interactions between different factors
Group 1 variables met the assumption of normal distribution, were analyzed by two-way Anova, and were provided with SEM. Group 2 variables did not meet the assumption thus, were analyzed by Permutation test and specified with IQR.

Data for observation assessment at 32, 42, and 52 weeks of hen age is shown in Table 7. Age, strain, housing environment, time of the day, and their interactions significantly affected some behavior repertoire of laying hens. The proportions of birds that were stretching ($p < 0.001$), pecking other objects ($p < 0.001$), walking ($p < 0.001$), standing ($p < 0.001$), and foraging ($p < 0.05$) decreased from 32 to 52 weeks. However, the proportions of birds preening were significantly higher ($p < 0.001$) at 52 weeks following a decreasing trend from 32 to 42 weeks. There was no age effect for proportions of birds that were wing flapping, drinking, feeding, pecking other hens, and dust bathing ($p > 0.05$).

The proportions of birds pecking other hens ($p < 0.001$), walking ($p < 0.05$), and standing ($p < 0.001$) were higher for S than W strain. However, the proportion of birds preening was higher for the W than S strain ($p < 0.05$). The proportions of birds that were wing flapping

**Table 7.** Observed behaviors (%) in laying hens due to housing environment, hen strain, and time of day when tested at 32, 42, and 52 weeks of age.

| Age of hens (A) | Wing flapping | Stretching | Preening | Drinking | Feeding | Pecking other hen | Pecking object | Dust bathing | Foraging | Walking | Standing |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 32 | 2.84 | 2.90[a] | 18.92[b] | 14.93 | 23.91 | 3.79 | 58.58[a] | 2.29 | 16.48[a] | 70.99[a] | 70.52[a] |
| 42 | 3.35 | 1.36[b] | 10.89[c] | 13.45 | 24.38 | 3.11 | 39.47[b] | 1.14 | 8.83[b] | 58.66[b] | 58.93[b] |
| 52 | 3.11 | 1.40[b] | 23.39[a] | 11.81 | 25.06 | 2.66 | 36.88[b] | 2.63 | 13.33[a] | 56.48[b] | 53.19[c] |
| **Housing environment (HE)** | | | | | | | | | | | |
| DL | 3.72 | 2.56 | 42.62[a] | 23.81[a] | 43.21[a] | 9.58[a] | 44.79 | 3.33 | 9.85[b] | 72.62[a] | 73.75[a] |
| PC | 3.61 | 1.81 | 14.86[b] | 10.42[b] | 21.81[b] | 1.67[b] | 45.97 | 1.25 | 13.33[ab] | 60.83[b] | 59.31[b] |
| MP | 2.22 | 1.67 | 10.28[b] | 11.53[b] | 17.50[b] | 1.53[b] | 44.03 | 1.81 | 16.94[a] | 59.03[b] | 57.22[b] |
| MS | 3.04 | 1.74 | 11.47[b] | 11.29[b] | 21.54[b] | 2.10[b] | 45.05 | 2.11 | 10.38[b] | 59.21[b] | 57.53[b] |
| **Hen strain (HS)** | | | | | | | | | | | |
| S | 4.08 | 1.71 | 15.65 | 14.19 | 23.80 | 5.54 | 46.09 | 2.59 | 14.00 | 64.87 | 64.03 |
| W | 2.11 | 2.07 | 19.82 | 12.60 | 25.11 | 0.83 | 43.86 | 1.44 | 11.76 | 59.21 | 57.73 |
| **Time (T)** | | | | | | | | | | | |
| Morning | 4.11 | 2.30 | 16.15 | 12.24 | 22.97 | 2.85 | 44.99 | 1.30 | 14.73 | 62.60 | 60.73 |
| Afternoon | 2.08 | 1.48 | 19.31 | 14.55 | 25.94 | 3.53 | 44.96 | 1.74 | 11.03 | 61.48 | 61.03 |
| **SEM** | 0.45 | 0.28 | 1.26 | 1.12 | 1.64 | 0.48 | 1.58 | 0.45 | 1.05 | 1.36 | 1.38 |
| **P values** | | | | | | | | | | | |
| A | 0.928 | <0.013 | <0.001 | 0.558 | 0.976 | 0.434 | <0.001 | 0.215 | <0.003 | <0.001 | <0.001 |
| HE | 0.277 | 0.595 | <0.001 | <0.001 | <0.001 | <0.001 | 0.971 | 0.454 | <0.049 | <0.002 | <0.001 |
| HS | 0.070 | 0.577 | <0.021 | 0.447 | 0.734 | <0.001 | 0.397 | 0.094 | 0.313 | <0.014 | <0.005 |
| T | <0.038 | 0.129 | 0.223 | 0.423 | 0.472 | 0.400 | 0.806 | 0.041 | 0.129 | 0.658 | 0.887 |
| A * HE | 0.391 | 0.644 | 0.322 | 0.601 | 0.444 | 0.513 | <0.004 | 0.535 | 0.053 | 0.391 | 0.569 |
| A * HS | 0.531 | 0.647 | 0.547 | 0.486 | 0.714 | 0.700 | 0.207 | <0.016 | 0.407 | 0.263 | 0.378 |
| A * T | 0.068 | 0.943 | 0.130 | 0.660 | 0.698 | 0.716 | 0.565 | 0.244 | 0.421 | 0.919 | 0.834 |
| HE * HS | 0.202 | 0.650 | 0.726 | 0.672 | 0.950 | <0.001 | 0.888 | 0.153 | 0.514 | 0.309 | 0.179 |
| HE * T | 0.680 | 0.719 | 0.274 | 0.992 | 0.741 | 0.728 | 0.437 | 0.057 | <0.048 | 0.913 | 0.989 |
| HS * T | 0.380 | 0.161 | 0.474 | 0.460 | 0.204 | 0.093 | 0.058 | 0.752 | 0.284 | 0.070 | 0.224 |
| A * HE * HS | 0.860 | <0.046 | 0.916 | 0.458 | 0.694 | 0.516 | 0.978 | 0.887 | 0.487 | 0.869 | 0.691 |
| A * HE * T | 0.740 | 0.714 | 0.718 | 0.950 | 0.492 | 0.951 | 0.916 | 0.229 | 0.764 | 0.683 | 0.690 |
| A * HS * T | 0.198 | 0.730 | 0.775 | 0.166 | 0.365 | 0.810 | 0.764 | 0.164 | 0.579 | 0.642 | 0.522 |
| HE * HS * T | 0.279 | 0.190 | 0.947 | 0.952 | 0.702 | 0.202 | 0.886 | <0.013 | 0.167 | 0.936 | 0.792 |
| A * HE * HS * T | 0.520 | 0.707 | 0.770 | 0.625 | 0.991 | 0.748 | 0.650 | 0.385 | 0.565 | 0.202 | 0.181 |

Abbreviations: DL: Deep litter, PC: *Petroselinum crispum,* MP: *Mentha piperita*, MS: *Medicago sativa*, S: Lohmann Sandy, W: Lohmann LSL Classic, SEM: Standard error of means, *: Interactions between different factors

Means within the same column with different letter superscript significantly differ ($p < 0.05$)

nearly reached a significant level ($p = 0.070$). No laying hen strain differences were observed for the proportion of birds that were stretching, drinking, feeding, pecking other objects, dust bathing, and foraging ($p > 0.05$).

The proportions of hens that were preening ($p < 0.001$), drinking ($p < 0.001$), feeding ($p < 0.001$), pecking other hens ($p < 0.001$), walking ($p < 0.01$), and standing ($p < 0.001$) were higher in the DL than in the outdoor environment groups. The proportion of hens that were foraging was highest in the MP environment than in other groups ($p < 0.05$). The proportions of birds that were wing flapping, stretching, pecking objects, and dust bathing were similar across the housing environments ($p > 0.05$).

The proportion of hens that were wing flapping was higher in the morning than afternoon and those dust bathing was higher in the afternoon than morning ($p < 0.05$). There was no difference in the proportions of birds that were stretching, preening, drinking, feeding, pecking other hens, pecking objects, foraging, walking, and standing across times of the day ($p > 0.05$).

The proportion of birds pecking objects was influenced by the age * housing environment interaction ($p < 0.01$). The proportion of those dust bathing was affected by age * hen strain and housing environment * hen strain* time of day interactions ($p < 0.05$). The housing environment * hen strain interaction affected the proportion of birds that were pecking at other hens ($p < 0.001$). The housing environment * time of day interaction had an impact on the proportion of birds that was foraging ($p < 0.05$). The age * housing environment * hen strain interaction influenced the proportion of birds stretching ($p < 0.05$).

## 4. Discussion

The current study showed that the age of hens did not significantly affect TI duration, which is not in line with Hocking et al. [30], who found an age-related effect on fearfulness in hens. The above authors reported that TI duration decreased with the age of hens. However, the decreasing trend in the current results would be consistent with the earlier established suggestion that TI duration is sensitive to repeated testing and so, declines with repeated testing of the same birds [31,32].

The results of the current study demonstrated that both the W and S strains are characterized by similar levels of fearfulness. This is supported by Hocking et al. [30], who reported no overall effect of hen strain on TI duration. However, this is contrary to several authors who determined that the duration of TI has a genetic basis [8–10,14].

In the present study, the housing environment did not affect the TI duration of hens. Similar results were determined by Campo et al. [33] when hens were housed in pens with or without access to the outdoors. Additionally, in the present study, the effect of the housing environment on the duration of TI nearly approached significant levels, being lower for outdoor plant housing environments. It can be speculated that specific plant characteristics, that is, height, density, and bioactive compounds could probably modify the personality traits and physiological developments associated with fearfulness in birds differently. The lack of effect of interactions on TI duration could possibly be a consequence of the absence of the effect of the main factors.

This study showed that total feather scores deteriorated with the aging of birds. Previous studies examining feather conditions have suggested similar results [18,21,34,35]. In the present study, hens in outdoor plant housing environments had the best feather coverage compared with deep litter hens. These results would be in line with Sokolowicz et al. [21], who observed the largest feather losses in hens kept in deep litter than in free-range systems. Moreover, there are suggestions that access to outdoor increases the bird's motivation to forage. This reduces feather pecking, resulting in less feather pecking and feather damage [14,35,36]. Also, the lowest feather scores in DL hens at 42 and 52 weeks might indicate that there is a pronounced increase in feather pecking behavior with an increase in the age of birds in completely indoor than in the outdoor-based systems.

The strain of laying hens affected the total feather score, which would be consistent with the previous studies [14,21,34]. These reports associate feather loss with feather pecking and aggression [22]. In this study, the behavior of pecking other hens was higher in the S strain accompanied by lower total feather scores than the W strain, demonstrating strain differences in feather condition due to genetic disparity in pecking of other hens, especially feather pecking and pulling.

In this study, footpad dermatitis was not detected in both strains regardless of age and housing environment. On the contrary, genetic variability in the occurrence of footpad dermatitis has been reported [14,19,20]. The results of the present study would suggest that there was better management of the housing conditions, especially litter quality in the deep litter areas during the study period. In the indoor environment, moist litter is the main risk factor for footpad dermatitis [5,6]. During the study period, litter was changed frequently ensuring that it was dryer and free from manure accumulation and caking, especially during the winter season. In the outdoor areas, the terrain was free of mud pools, one of the risk factors for footpad dermatitis in outdoor systems, because the study region is characterized by low rainfall and the soils, have a low water holding capacity.

The comb surface, breast region surface, footpad surface, and rectal temperatures were lowest at 42 weeks of

age. This is probably associated with the seasonal effect (i.e. wider fluctuation in ambient temperatures). The reason is that the sampling times, at 31, 42, and 52 weeks of hen's age occurred in autumn, winter, and spring, respectively. The variation in body region temperatures at different ages would indicate the bird's ability to respond to various stressors, especially the changes in ambient temperatures [23,37,38].

The housing environment did not affect all the measured body region temperatures. This partially agrees with previous studies that found no differences in rectal temperatures in broilers, where the chickens were housed with or without access to vegetated areas [39, 40].

In the present study, the hen strain difference concerning comb and rectal temperature was identified. A study by Van Kampen [37] provided evidence for the significant role of the comb in heat dissipation. The present results could probably suggest a genetic influence on regulating heat dissipation from the comb. On the other hand, there was a lack of hen strain difference for the remaining body region temperatures. A similar effect was observed by Mutaf et al. [41] when they assessed the core body, head surface, and dorsal surface temperatures in Atak-S (brown hens) and Atabey (white hens). The similarity in some body region surface temperatures between hen strains could probably suggest that both strains might have been developed from breeds with a similar ability to cope with changes in environmental temperatures.

The typical natural behaviors can include dust bathing, foraging, and forms of pecking (pecking other hens and pecking objects) [27]. The current study demonstrated age effect on some natural behaviors, that is, the proportion of hens expressing pecking objects. This agrees with Campbell et al. [29], who identified age-related effects on some natural behaviors, except for dust bathing and foraging behaviors.

There are reports that natural behaviors can be expressed more outside than inside [36, 42]. However, the vegetation type or topography outside can play an important role [43]. This is partially consistence with the present study where the proportion of hens pecking other hens was higher in MS, PC, and MP than in DL hens. Similarly, Oke et al. [39] found a lower frequency of spot pecking and feather pecking in deep litter broilers than those allowed access to different pasture species and free run without vegetation. Nonetheless, in their study, dust bathing was expressed more in outdoor than in deep litter, contradicting the current results. In this study, foraging behavior was higher in MS than in PC and MP housing environments. This would highlight that the plant characteristics (i.e. height, density, and bioactive compounds) might modify the physical and physiological development related to behavioral development in early life and behavioral expression in late life of birds [29].

In this study, the strain disparity in the behavior of pecking of hens is in support of Hocking et al. [30], who reported genetic influence on feather pecking. Furthermore, feather pecking is described as redirected foraging. It is reported that genetic differences in the development of feather pecking would depend on genetic differences in foraging behavior [44,45]. On the contrary, strain difference in foraging behavior was not observed in the present study, which is not in correspondence with Klein et al. [44]. This would be due to the discrepancies in the description of foraging behavior between studies. Additionally, there was no variation in other natural behaviors between strains in this study. This would not be in agreement with Riddle et al. [46] and Sokołowicz et al. [47], who determined that dust bathing differed among laying hen strains.

In the present study, the age and housing environment interaction effect on the behavior of pecking objects reflects the bird's response to the housing environment due to the biological changes that happen with aging, experience, maturity, and season of the year. Also, the hen strain and housing environment interaction effect on the proportion of birds pecking other hens indicates that the tendency to feather peck strongly depended on how the strains responded to the conditions of the different housing environments.

Wing flapping, stretching, and preening are always classified as comfort behaviors in birds and are associated with body maintenance. The current study indicated age-related effects in the proportion of birds that were preening and stretching but not wing flapping. Age-related effects on comfort behaviors in free-range hens were also reported by Sokołowicz et al. [47]. In the present study, the housing environment was only observed on preening and higher in deep litter hens, which would not be in line with several studies [39,47]. These authors reported that expression of comfort behaviors was higher in outdoor than indoor hens. In this study, it was shown that the hen strain effect was only observed on preening behavior, although it nearly reached significant levels for wing flapping. This would probably indicate genetic influence on some comfort behaviors as reported by Riddle et al. [46], who found that wing flapping varied among the four laying hen strains. However, Sokołowicz et al. [47] observed a genetic influence on the expression of all the comfort behaviors.

The findings of the current study indicated that the proportion of birds performing feeding and drinking behaviors was similar across the age of hens. This does not agree with Sözcü et al. [48], who found an increase in drinking and feeding behavior with an increase in the age of birds. The effect of the housing environment on

drinking and feeding behaviors is in accordance with Oke et al. [39] and Ipek and Sözcü [40], who identified that feeding behavior was lower in free-range birds than deep litter birds. It seems the vegetation outside could be a form of environmental enrichment that facilitates birds to perform other physical activities than feeding and drinking. However, the similarity between hen strains would not agree with the research by Sözcü et al. [48], who determined a higher percentage of hens feeding and drinking in Atak-S (brown) than Atabey (white) strain when kept in a free-range system.

A declining trend was observed in the proportion of birds walking and standing. This age effect on walking and standing would be ascribed to reduced activities such as area of space use covered, and distance moved with the aging of birds [49]. In the current study, the proportion of birds walking and standing was higher in deep litter than in outdoor plant housing environments. This could probably indicate that the vegetation outside motivated the birds to engage in other activities than walking and standing. The hen strain differences observed in walking and standing could indicate that different strains are characterized by different kinetic energies due to disparity in genetic make-up and greater variability in vigilance behaviors [50]. In agreement with the present study, some previous studies also determined genetic influence on standing and walking behaviors [48,51].

The results of the present study indicated that the age of hens did not affect the H/L ratio. This would conflict with Lentfer et al. [17], who determined that mean H/L ratios of hens in the Rihs bolegg II and Volito voletage aviary systems differed at different ages. Also, the housing environment did not affect blood parameters. The results are not in accordance with Campo et al. [33], who reported significantly greater H/L in deep litter than in free-range hens. Additionally, Moe et al. [15] and Yilmaz Dikmen et al. [52] also identified significant variations in H/L ratios based on the housing system. Generally, the difference between the results of the current study and the previous studies could be associated with the ages when blood samples were taken, strain differences, and probably the specific type of housing environment. In the present study, blood parameters were not significantly influenced by hen strain. This is not consistent with several studies that observed strain differences in relation to some blood parameters, especially the H/L ratio in birds due to long-term variations in the environment [11–17]. The current results suggest that the strains that were used in the study might show similar ranges of immune responses in reaction to stress stimulus [20].

## 5. Conclusions

The present study was able to determine that feather loss is increased in hens housed completely indoors than in those with access to the outdoor environment. Additionally, feather loss is reduced for the W than the S strain. Also, the comb surface temperature may be a heritable trait since it was higher for the S than the W strain. Furthermore, the proportion of hens preening, feeding, drinking, pecking other hens, walking, and standing behaviors is increased in the DL than with access to outdoor plant-based environments. The proportion of hens' wing flapping and foraging is reduced and increased, respectively with access to MP and for those pecking objects is increased with access to PC. Pecking other hens, walking, and standing behaviors are more expressed by the S than the W strain. However, preening behavior is more expressed by the W than S strain. Meanwhile, feather loss is increased with the aging of hens. Also, there are age-related effects on body region temperatures of hens, and expression of preening, stretching, pecking objects, foraging, walking, and standing. In addition, most of the hens express wing flapping and dust bathing in the morning and afternoon, respectively. This study demonstrates greater variability in feather condition and the expression of some behaviors due to the housing environment and laying hen strain. However, further studies are warranted to refine the influence of housing environment and hen strain on the duration of tonic immobility, body region temperatures, foot health, and blood parameters.

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