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Original Article

Evaluation of Production Performance Parameters of Two Laying Hen Strains Housed in Four Housing Environments

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ABSTRACT

This study investigated the impact of housing environment on performance parameters of the Lohmann LSL Classic (LW) and Lohmann Sandy (LS) strains. The deep litter (DL), free access to outdoor *Mentha piperita* (MP), *Petroselinum crispum* (PC), and *Medicago sativa* (MS) housing environments were examined. A total of 260 four-week-old birds were randomly allotted to DL and outdoor plant-associated groups, with four and three replicates, respectively, and 10 birds per replicate. Data collection for weekly feed intake began at 4 weeks until the end of the study. Weekly hen weights were measured from 4 weeks of age until the age of 50% egg production; and then later, at 52 weeks of age. From the day of the first egg until the last day of the study, eggs laid in each nest box tier (upper, middle, bottom, and floor) were recorded and weighed at 9:00 a.m., 12:00 noon, and 3:00 p.m. Housing environment influenced the age at first egg and 50% egg production, hen-day egg production, feed intake during the egg-laying period, feed conversion ratio, average egg weight, and egg size distribution ($p<0.01$; $p<0.05$). Age at first egg and 50% egg production, hen-day egg production, feed intake, average egg weight, and egg size distribution varied between strains ($p<0.01$; $p<0.05$). Most of the eggs were laid in the morning and on the upper nest box tier ($p<0.01$). Additionally, average egg weight varied among the egg-laying time and nest box tiers, and most of the eggs laid were in the large egg size (56-65 g) category ($p<0.01$). It was concluded that access to *Medicago sativa* offers hens the most ideal environment for production performance. The two strains were quite similar in terms of overall performance outcomes.

INTRODUCTION

The publication in 1964 of "Animal Machines", by Ruth Harrison (Van de Weerd & Sandilands, 2009), and subsequently the Brambell report in 1965 (Brambell, 1965) increased consumer awareness about the quality of life of chickens in various housing systems. Since then, consumer-driven policies and legislation in the layer chicken industry, especially concerning housing systems, have accelerated in many developed countries (Tainika & Şekeroğlu, 2020). The standout policies have involved the accelerated transition to "animal-friendly" or "welfare-friendly" production systems, referred to as cage-free systems. However, designing these systems brings challenges, notably that of balancing the health and welfare of hens with consumer preferences, the needs of the egg industry, and increased costs of management (Anderson, 2014).

It is understood that there can be considerable variations between housing systems or environments in relation to performance indicators such as age at first egg, age at sexual maturity, body weight, hen house and hen day egg production, feed intake, feed conversion ratio, and mortality (Küçükyılmaz *et al*., 2012; Yılmaz Dikmen *et al*., 2016; Ketta *et al*., 2020; Rakonjac *et al*., 2020; Racevičiūtė-Stupelienė *et al*., 2023). The hen strain also has a significant impact on the production performance traits mentioned above (Küçükyılmaz *et al*., 2012; Ketta *et al*., 2020; Rakonjac *et al*., 2020).

Meanwhile, country-based egg size categories are important marketing and quality tools for producers. Earlier studies on the performance of laying hens have not always evaluated this aspect. Recently, Alig *et al*. (2023a, 2023b) reported that there are differences in egg size distribution based on hen strain and housing system.

Egg-laying time is also an important performance trait of laying hens, mainly due to its influence on some egg quality parameters (Tůmová & Gous, 2012; Tůmová & Ebeid, 2005). Egg-laying time in hens is influenced by photoperiod (Lewis *et al*., 1995, 2004; Campo *et al*., 2007). Several studies have determined that most of the eggs are laid in the morning, and egg-laying time can vary between different hen strains and hen ages (Tůmová & Gous, 2012; Tůmová & Ebeid, 2005; Tůmová *et al*., 2017a).

It is fully established that laying hens should be provided with laying nest boxes to satisfy their behavioural needs (Weeks & Nicol, 2006). While most studies have focused on laying nest box size, location, and material (Cooper & Appley, 1996; Ringgenberg *et al*., 2014), it is still not fully investigated whether the nest box tier matters to laying hens in cage-free systems. However, a few studies found influences of genetics (Krause & Schrader, 2018), and housing environment on hens' nest height preferences (Appleby *et al*., 1986; Bari *et al*., 2020).

In past decades, it has been advised that free-range hens should be granted access to outdoor-vegetated areas (Hammershøj & Johansen, 2016). Several reports have revealed that access to outdoor legume pastures can enhance some performance indicators in hens (Oke *et al*., 2016; Kop-Bozbay *et al*., 2021). This means that what is consumed by the birds is one of the main determining factors in performance measurements, and not whether hens are allowed range accessibility or not (Hammershøj & Steenfeldt, 2005; Horsted & Hermansen, 2007; Hammershøj & Steenfeldt, 2012; Steenfeldt & Hammershøj, 2015; Hammershøj & Johansen, 2016). Moreover, aromatic plant species are nutritional and available, and have been commonly tested as feed additives for chickens. However, there is almost no information about the effect of access to outdoor areas vegetated with aromatic plant species on the performance of free-range laying hens.

In general, more research is still needed on how access to different outdoor plant species impacts performance in hens, especially where plant species vary in nutritional value and palatability to hens (Horsted *et al*., 2007). Meanwhile, in the past decades, the dynamics in housing systems for hens have also influenced layer chicken breeding strategies, whereby the breeder companies have focused on breeding strains that can cope with the conditions of alternative production systems (Lohmann, 2022). Nevertheless, modern commercial laying hen strains may differ in terms of range use and adaptation to outdoor features.

Thus, this study evaluated the effects of housing environments (deep litter without or with access to outdoor *Mentha piperita*, *Petroselinum crispum,* or *Medicago sativa*) and genetic strains (Lohmann LSL Classic and Lohmann Sandy) on production performance indicators of laying hens. It was hypothesized that hens reared in deep litter with outdoor access to pasture plants would have better performances compared to those reared in deep litter without outdoor access, and that the Lohmann LSL Classic strain would have better performance than the Lohmann Sandy strain.

MATERIALS AND METHODS

Ethical approval

This study followed the guidelines that regulate animal experiments of the Ministry of Food, Agriculture and Livestock, Türkiye. Approval was granted by the animal experiments local ethics committee of Niğde Ömer Halisdemir University (approval number: 2021/04).

The trial was conducted from June 2022 to May 2023 at the Ayhan Şahenk Agricultural Application and Research Centre of Niğde Ömer Halisdemir University (37 ° 58′ North 34 ° 40′45 East, elevation; 1299 m) in Niğde province, Türkiye.

Establishment of experimental plants

Prior to sowing, soil preparation through ploughing, removal of stones, and levelling in each outdoor pen was carried out for each outdoor pen measuring 9.41 m × 1.94 m (total area = 18.25 m^2). There was no herbicide treatment because it was not recommended by the university. Then, the pens were randomly assigned to the studied plant types: mint (*Mentha piperita*), parsley (*Petroselinum crispum*), and alfalfa (*Medicago sativa*).

Mentha piperita was cultivated by vegetative propagation utilizing suckers or sprigs. The suckers were first grown in pots in the greenhouse, then transferred to experimental plots with a well-developed shoot system. During the establishment, to ensure a dense plant cover, 200 suckers of *Mentha piperita* were planted in a square meter. The suckers were planted in rows and columns, leaving a space of 10 cm between each of them.

Petroselinum crispum and *Medicago sativa* were established utilizing seeds acquired from a certified seed dealer in the province. The proposed seeding rates were 183 g per pen (corresponding to 10 g of seeds per square meter) for *Petroselinum crispum* and 275 g per pen (resulting in 15 g of seeds per square meter) for *Medicago sativa*. As a note, one kg of artificial fertilizer was first added to the seeds of both plants, and subsequently spread by hand across their respective planting areas.

Moreover, the sowing of *Petroselinum crispum* seeds was followed by covering the soil with sacks in the planted pens until germination. This was determined by a professor experienced in forage plants, who highlighted that seeds for *Petroselinum crispum* can dry (no germination) when left exposed to direct sunlight. The sowing process was carried out across two consecutive days, and often late in the evening.

The planted fields were then irrigated by sprinklers twice each day (morning and evening) until the suckers were established and seeds sprouted. Thereafter, the method of watering was switched to flood irrigation (once a day) until the complete plant coverage of pens. During the growth stage of plants, weeds were often pulled out as soon as they were identified. The plant coverage of the various species was maintained at the vegetative stage by regular mowing.

Birds, housing, and management

Two egg-laying strains, Lohmann LSL Classic (LW) and Lohmann Sandy (LS), were used for this study. A total of 300 (n = 150 for each strain) beak-trimmed three-week-old chicks belonging to the same breeder company were brought to the experimental unit and reared in two different deep litter pens based on the strain for seven days. This was aimed at familiarizing the chicks with the new environment. These strains were selected based on the estimated level of popularity in the egg industry: while the LW strain is well-recognized worldwide for its efficient production of white eggs, the LS is not yet common in the egg industry. The LS hybrid line possesses white feathers, lays cream-colored eggs, and is marked by a good feed conversion ratio and vigour (Lohmann, 2022).

At 4 weeks of age, the chicks were individually weighed and placed randomly in the replicate pens corresponding to the housing environment in which they would be kept. The housing system pens were determined prior to the establishment of plant compositions. The birds were separated between the deep litter system without outdoor access (DL), and deep litter with access to either *Mentha piperita* (MP), *Petroselinum crispum* (PC), or *Medicago sativa* (MS). For each strain, the number of replicates per rearing environment was four for DL and three for plant-associated groups, each with a total of 10 birds. Overall, a total of 26 replicate pens and a total of 260 birds were utilized for the study, and the experimental groups were DLW, DLS, MPW, PCW, MSW, MPS, PCS, and MSS. All the housing environments were in the same poultry house.

During their 11 weeks of age, all the birds were reared completely indoors. The total area of each indoor pen was 2.79 m^2, comprising a layer of wheat straw as litter material, maintained at a depth of 8 cm from the concrete floor and wire mesh walls. In each indoor pen, there was one hanging feeder (41 cm in diameter) and a round bell drinker (30 cm in diameter), and hens had *ad libitum* access to feed and water. The units for the DL system were 16 in total and were located in the centre of the poultry house. However, only eight pens (four replicate pens per strain) close to the entrance and exit of the poultry house were used for this study. DL birds were kept completely indoors up to the end of the study, at 52 weeks of age,. The remaining eight DL pens located in the middle were left empty. A corridor of almost three meters wide separated the DL and plant-related housing environments on both sides of the poultry house.

Indoor stocking density was 3.58 birds / m^2 and did not consider the area occupied by the drinker, feeder, and nest box. Additionally, there was a 3 × 3 (tier and cell) metallic nest box that measured 98 cm × 37 cm × 138 cm from 19 weeks of age. The different nest box tiers were 94 cm, 59 cm, and 24 cm off the ground level. There were also two horizontal landing platforms permanently fixed 4 cm below and in front of each nest box tier. During the study, new litter was added every time its quantity reduced, and litter was changed each time caking was discovered.

On the other hand, all the indoor pens for plant-based housing systems (MP, PC, and MS) had a pop hole (50 cm high × 50 cm wide) in the centre that allowed birds access to contiguous outdoor pens. The total area of each outdoor unit was 18.25 m^2 and comprised either *Mentha piperita*, *Petroselinum crispum*, or *Medicago sativa*. The pop holes were regularly opened each day from 8:30 a.m. to 3:30 p.m., from 12 weeks of hens` age until the end of the study, to allow for the birds to access the range. The plants were at a uniform height of 20 cm on the first day the birds were granted access to the range. The stocking density of birds on the range was 0.55 birds / m^2 (outdoor area of 1.825 m^2/ bird). Outdoor pens were enclosed and divided by a wire mesh, preventing the crossover of birds from one pen to another. Due to the lack of a net covering the top, some specific birds (n = 8) could fly to the adjacent pens. To prevent this, the flight feathers of one wing of these birds were trimmed before placement in their respective pens.

During the study, the forage quality was preserved by watering each day in the evening when the pop-holes were closed (3:30 p.m.). Weeds were consistently removed from the range areas with the aim of reducing their effects on other plant species and the birds. Other forage quality preservation measures such as rotational foraging, fertilizer application, and mowing were not employed during the entire period of bird's access to the range.

Furthermore, during the study, we observed a variation in plant coverage which was mostly linked to changes in climatic conditions (the shift towards winter weather), and to a lesser extent the activities of birds. Between August and September, the outdoor pens had dense plant coverage. The coverage decreased to approximately 60% in October, 30% in November, until there was almost no plant coverage in December, and absolutely no coverage other than the standing stem parts without leaves from January to February. Plants began to grow again in March.

As soon as the chicks arrived, electric heaters were turned on and indoor temperature was maintained at 23°C until they were completely feathered. It was also thought that maintaining the above temperature could aid the birds to cope with the ambient temperatures outdoors. Nevertheless, from 8 weeks of age, the heaters were turned off and the indoor temperatures were free to fluctuate until the end of the study. In case of elevated indoor temperature, automatic fans were turned on to cool the environment. Throughout the study, no indicators of heat stress in birds were observed. Furthermore, due to extremely low temperatures in winter (February) (n = 21 days), the electric heaters were turned on and the pop-holes remained closed, considering the welfare of the birds.

The birds used in the study were offered commercial concentrate layer feed purchased from a certified feed manufacturer in accordance with their age, as shown in Table 1.

All the birds were provided with the same lighting program (light, L to dark, D) throughout the duration of the study following standard industry recommendations. In summary, at three weeks of age 13L:11D was offered, followed by a step-down program until 17 weeks of age according to the Lohmann breeder guide (Lohmann, 2022). Subsequently, the lighting program was altered based on the age of the birds: 10L:14D at 18 weeks, 10.30L:13.30D at 19 weeks, 11.15L:12.45D at 20 weeks, 12L:12D at 21 weeks, 12.45L:11.15D at 22 weeks, 13.30L:10.30D at 23 weeks, and 14L:10D at 24 weeks. The lighting cycle was then increased by 30 minutes each week, and at 27 weeks of age, the photoperiod reached 16L:8D, which was maintained throughout the duration of the study.

Table 1 – Composition of concentrate feed at various ages 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 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. The premix vitamin and mineral per kg of the diet: 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.

Under the 16L:8D lighting schedule, the lights were automatically switched on at 6:00 a.m. and switched off at 10:00 p.m. daily. Lighting provided using warm white LED bulbs of 14 watts / 2700 K. The light bulbs were cleaned with a cloth when necessary, to prevent the buildup of dust.

From day 0 to three weeks of age, birds were vaccinated at the breeder firm following their guidelines. At 11 and 23 weeks of age (during the study), the birds were vaccinated against Infectious bronchitis and Newcastle (Ma5 + Clone30 in drinking water), and fowl pox (VAIOL - VAC via wing web), respectively. Also, a mixture of vitamins and amino acids via drinking water was often offered to the birds after the vaccination process.

It is highlighted that the study happened between June 2022 and May 2023, which caused a wide variation in weather. At the time the pop-holes were opened (12 weeks of hen age), outdoor temperatures ranged from 23 - 35°C in August, then ranging from 8 - 34°C between September and November, 3 - 19°C between December and February, and finally 9 - 25°C between March and May. Indoor temperatures ranged from 20.9 - 31°C in August, from 5.6 - 30.3°C between September and November, from 1.1 - 15.1°C between December and February, and from 4 - 20.1°C between March and May

DATA COLLECTION

Production performance measurements

Hen weights (HW, g). The weekly HW per replicate pen from the 4 weeks of age until the age of sexual maturity (50% egg production) and on the final day of the study were determined by weighing individual hens with a 0.1 g precision balance.

Feed intake (FI). FI of each group was determined on a weekly basis based on the total feed consumption (g/ bird) and average gram of feed/ hen considering two periods: from 4 to 20 weeks (rearing period) and from 21 to 52 weeks of age (egg-laying period). Feed intake during each period was calculated as follows:

$$FI = \left(\frac{\textit{total grams of feed consumed during the period}}{\textit{hen days during the period}}\right)$$

Weekly feed intake was determined by weighing the remaining feed in the feeders with a precision balance of 0.1 g, which was then subtracted from the total feed given throughout the week.

Feed conversion ratio (FCR). FCR for each group was determined by dividing the total feed weight given to the animals during the egg-laying period by the total weight of all eggs that were obtained from each replicate pen.

$$FCR = \left(\frac{\textit{total feed weight of each group during the egg – laying period}}{\textit{total egg weight during the egg – laying period}}\right)$$

Liveability (Liv). The daily mortalities were recorded, and later Liv was determined separately for the two periods: the rearing period (4-20 weeks), and the egg-laying period (21-52 weeks). Liv was calculated as follows: (Number of hens at the beginning of each period – number of hens remaining at the end of each period)/ (number of hens at beginning of the period)) × 100.

Age of first egg (AFE, days): was indicated as the day the first egg was collected from the nest box.

Age at sexual maturity (ASM, days): was determined as the day when the hens reached 50% of egg production for each replicate pen.

In all the replicate pens, laid eggs were collected and recorded three times per day: 9:00 a.m., 12:00 noon, and 3:00 p.m., with an emphasis on the nest box tier (being designated as upper, middle, bottom, and floor eggs) from the day of the first egg till the end of the study (52 weeks).

Hen day egg production (HDE): was calculated as follows:

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

Hen-housed egg production (HHE): was determined using the formula below.

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

Average egg weight (AEW)

All the eggs collected from each replicate pen every day up to 52 weeks of hen age were also individually weighed on the same day in accordance with the collection time and nest box tier, as stated above. Average egg weight (AEW) was calculated as the total grams of eggs weighed divided by the number of eggs weighed.

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

Later, the weights of individual eggs per replicate pen were segregated into the USDA egg size categories (USDA 2000): small (42.0-49 g; Sm), medium (49-56 g; M), large (56-65 g; L), extra-large (65-70 g; XL), and jumbo (≥ 70 g; XXL). The classification of eggs in each

USDA egg size group was expressed as a percentage of the total number of eggs collected from each treatment replicate pen.

Egg-laying time and nest box tier preference

Egg-laying time and nest box tier preference were determined based on the percentage of eggs recorded at each egg collection time and in each nest box tier.

Statistical analysis

Data analysis involved two subgroups. In the first subgroup, the effect of the factors on the growth and reproductive performance-related characteristics of hens was examined. In the second subgroup, the effect of factors on AEW was investigated. Since the effects of each treatment group in the second subgroup were not replicated in the other treatment group, interaction effects were not examined. Therefore, a linear model was used for data analysis. Additionally, the weights of individual eggs were classified according to USDA criteria, and whether the distribution of this classification was dependent on environmental factors was investigated using chi-square analysis. The normality assumptions of the data were examined using the Kolmogorov-Smirnov test, and the Levene test was used to check the homogeneity of variance, confirming that the assumptions were met. Accordingly, an analysis of variance was then applied to the data. Intra-group multiple comparisons were made using the Duncan multiple comparison test. The statistical software package SPSS 21 was used for data analysis for both subgroups (IBM, 2012). The following statistical models were used for the analysis of the data:

First model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

In the model, Y_{ijk}: observation value, μ: population mean, α_i: the effect of the i. housing environment, β_j: the effect of the j. strain, $\alpha\beta_{ij}$: the effect of interaction and ε_{ijk}: the effect of random error $\varepsilon \sim N(0,\sigma^2)$.

Second Model:

$$Y_{ijklmn} = \mu + \alpha_i + \beta_j + \theta_k + \gamma_l + \delta_m + \varepsilon_{ijklmn}$$

In the model, Y_{ijklmn} :observation value, μ: population mean, α_i: the effect of i. strain, β_j: the effect of the j. housing environment, θ_k:the effect of k. age, γ_l: the effect of l. egg-laying time, δ_m: the effect of m. nest box tier and ε_{ijklmn}: random error; $\varepsilon_{ijklmn} \sim N(0,\sigma^2)$.

RESULTS

Results of average HW are shown in Table 2. Average HW statistically differed across housing environments only at week 19 of age; MS, PC, and MP hens were heavier than the DL hens ($p<0.01$). LS strains were significantly heavier than LW strains across the study ($p<0.01$).

Table 2 – Influence of housing environment and laying hen strain on hen weight, g at different ages (weeks).

		HW (g) at week 4	HW (g) at week 12	HW (g) at week 19	HW (g) at ASM	HW (g) at week 52
HE	MS	191.43	998.12	1388.60^b	1511.10	1794.10
	PC	190.68	993.68	1375.72^b	1503.47	1824.83
	MP	191.88	1001.75	1376.30^b	1504.73	1774.78
	DL	190.34	988.19	1283.43^a	1537.36	1769.13
	p value	0.503	0.755	<0.001	0.598	0.653
Hen strain	LS	195.75	1061.86	1454.31	1579.23	1915.44
	LW	185.94	927.89	1246.55	1452.67	1662.64
	p value	<0.001	<0.001	<0.001	<0.001	<0.001
	SEM	1.013	14.078	23.743	16.336	30.747
	HE × HS	0.704	0.734	0.222	0.261	0.098

Abbreviations: HW; hen weight, HE; housing environment, DL: deep litter, PC: *Petroselinum crispum*, MP: *Mentha piperita*, MS: *Medicago sativa*, LS: Lohmann Sandy, LW: Lohmann LSL Classic, SEM: standard error of means, x: interactions between different factors, HW; hen weight, ASM; age at sexual maturity.

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

Table 3 shows AFE, ASM, Liv, HHE, HDE, FI, and FCR data. It was observed that MS, PC, and MP hens laid the first egg and reached sexual maturity earlier than those in the DL environment ($p<0.001$). Also, AFE and ASM were earlier in the LS than the LW strain ($p<0.01$). Liv across the two periods (rearing and egg-laying) was not significantly different among the housing environments and between the hen strains ($p>0.05$). This study found that the housing environment and hen strain had no significant effect on overall HHE ($p>0.05$). However, the overall HDE significantly differed among the housing environments; being the highest and lowest for MS and DL birds, respectively ($p<0.001$). Also, overall HDE was higher for the LS than the LW strain ($p<0.01$).

While the housing environment had no significant effect on FI during the rearing period, it influenced FI during the egg-laying period; FI was lower for MS and MP hens than for PC hens, whose quantity was lower than that of DL hens ($p<0.01$). Hen strain had a significant effect on FI during both periods ($p<0.001$), with lower FI for the LW than the LS strain. A housing environment x hen strain interaction effect on FI during the rearing period was also observed ($p<0.01$).

FCR differed among the housing environments ($p<0.01$), whereas MS and MP hens had a lower (better) FCR than PC hens, whose value was better than DL hens. However, FCR was similar between the hen strains ($p>0.05$).

Table 3 – Influence of housing environment and laying hen strain on growth and reproductive performance-related parameters.

		AFE, days	ASM, days	4 - 20 weeks liv, %	21 - 52 weeks liv, %	Overall HHE, number of eggs	Overall HDE, number of eggs	4 - 20 weeks FI, g/bird	21 - 52 weeks FI g/bird,	FCR
HE	MS	125.17[a]	134.83[a]	98.33	98.33	221.53	224.55[c]	6773.17	30784.67[a]	2.29[a]
	PC	125.00[a]	134.50[a]	100	100	222.70	222.70[bc]	6800.33	31236.00[ab]	2.35[ab]
	MP	128.00[a]	135.50[a]	100	100	219.97	219.97[b]	6834.83	30724.83[a]	2.30[a]
	DL	137.00[b]	144.00[b]	96.25	100	206.10	214.51[a]	6635.50	32093.00[b]	2.44[b]
	p value	<0.001	<0.001	0.656	0.355	0.129	<0.001	0.142	<0.038	<0.022
Hen strain (HS)	LS	126.08	135.08	99.23	99.23	220.76	222.15	7077.92	31993.85	2.39
	LW	132.77	140.38	97.69	100	212.89	217.80	6424.69	30561.31	2.32
	p value	<0.008	<0.005	0.686	0.288	0.256	<0.012	<0.001	<0.002	0.086
	SEM	1.590	1.217	1.203	0.385	3.029	1.162	73.259	274.518	0.022
	HE × HS	0.147	0.477	0.451	0.355	0.258	0.062	0.525	<0.011	0.347

Abbreviations: HE: housing environment, DL: deep litter, PC: *Petroselinum crispum*, MP: *Mentha piperita*, MS: *Medicago sativa*, LS: Lohmann Sandy, LW: Lohmann LSL Classic, SEM: standard error of means, x: interactions between different factors, AFE: age at first egg, ASM: age at sexual maturity (50% egg production), Liv: liveability, HHE: hen-house egg production, HDE: hen-day egg production, FI: feed intake

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

The data for the AEW is shown in Table 4. AEW significantly increased with the aging of hens ($p<0.01$). Also, AEW significantly differed among the housing environments ($p<0.01$), with DL hens laying heavier eggs than hens in the MS, PC, and MP housing environments. Additionally, among the outdoor plant housing environments, MS hens laid the heaviest eggs. There was a significant difference in the AEW between the hen strains ($p<0.001$), which was higher for LS than LW hybrids. There was a decreasing trend in AEW across the egg-laying time ($p<0.001$), which was highest at 9:00 a.m. and lowest at 3:00 p.m. Also, there was an increasing trend in AEW, with an upward increase in the nest box tier ($p<0.01$): it was lowest for floor eggs and highest for upper nest box tier eggs.

Table 4 – Influence of laying hen strain, housing environment, hen age, egg-laying time, and nest box tier on average egg weight (g) during the entire study.

		Average egg weight	*p* values
Hen strain	LS	60.966	<0.001
	LW	60.976	
Housing environment	MS	60.680[b]	<0.001
	PC	60.349[c]	
	MP	60.709[b]	
	DL	61.908[a]	
Hen age	17-32	56.766[c]	<0.001
	33-42	63.213[b]	
	43-52	64.411[a]	
Egg-laying time	9:00	61.381[a]	<0.001
	12:00	60.683[b]	
	3:00	56.077[c]	
Nest box tier	Floor	58.082[d]	<0.001
	Bottom	60.191[c]	
	Middle	60.750[b]	
	Upper	61.860[a]	
SEM		0.026	

Abbreviations: DL: deep litter, PC: *Petroselinum crispum*, MP: *Mentha piperita*, MS: *Medicago sativa*, LS: Lohmann Sandy, LW: Lohmann LSL Classic, SEM: standard error of means. Means within the same column with different letter superscript significantly differ ($p<0.05$).

Overall, hens laid more L eggs than XL eggs and more M eggs than XXL eggs. Sm eggs were the lowest for all the groups (Table 5). With regards to age (Table 5), there was an increasing trend in the XL and XXL eggs and a decreasing trend in Sm, M, and L eggs with the aging of hens ($p<0.01$). Also, the number of eggs decreased with the increase in the age of hens, with more eggs laid from 17 to 32 weeks than from 33 to 42 weeks, the ratio of which was much higher than from 43-52 weeks. The USDA egg size distribution was influenced by the housing environment (Table 5; $p<0.01$): DL hens laid more L, XL, and XXL eggs compared with other groups. In terms of the outdoor plant housing environments, PC hens laid most L eggs but the least XL eggs, and MP laid the highest amount of XXL eggs. Strain affected USDA egg size distributions (Table 5; $p<0.01$), with the LS strain laying more Sm eggs and XXL eggs than the LW strain, and more L eggs being laid by the LW than the LS strain.

Table 5 – Influence of hen age, housing environment, and laying hen strain on USDA egg size distribution expressed as the number of eggs and ratio of eggs per category (%).

Parameter		USDA egg size distribution					
		Sm	M	L	XL	XXL	Total
	Overall, n	1818 (3.3%)	7735 (13.8%)	31181 (55.8%)	12069 (21.6%)	3072 (5.5%)	55875 (100)
Hen age (wks)	17-32	1806 (3.2%)	7130 (12.8%)	11974 (21.4%)	1091 (2%)	396 (0.7%)	22397 (40.1%)
	33-42	8 (0%)	446 (0.8%)	10877 (19.5%)	5072 (9.1%)	1105 (2%)	17508 (31.3%)
	43-52	4 (0%)	159 (0.3%)	8330 (14.9%)	5906 (10.6%)	1571 (2.8%)	15970 (28.6%)
	x^2 (P)	17824.2 (<0.001)					
HE	MS	362 (0.6%)	1818 (3.3%)	7708 (13.8%)	2586 (4.6%)	459 (0.8%)	12933 (23.1%)
	PC	467 (0.8%)	2013 (3.6%)	7874 (14.1%)	2353 (4.2%)	576 (1%)	13283 (23.8%)
	MP	484 (0.9%)	2009 (3.6%)	7063 (12.6%)	2972 (5.3%)	614 (1.1%)	13142 (23.5%)
	DL	505 (0.9%)	1895 (3.4%)	8536 (15.3%)	4158 (7.4%)	1423 (2.5%)	16517 (29.6%)
	x^2 (P)	889.024 (<0.001)					
Hen strain	LS	1071 (1.9%)	3890 (7%)	15527 (27.8%)	5986 (10.7%)	1761 (3.2%)	28235 (50.5%)
	LW	747 (1.3%)	3845 (6.9%)	15654 (28%)	6083 (10.9%)	1311 (2.3%)	27640 (49.5%)
	x^2 (P)	118.9 (<0.001)					

Abbreviations: wks: weeks, Sm: small (42.0-49 g), M: medium (49-56 g), L: large (56-65 g), XL: extra-large (65-70 g), XXL: jumbo (≥ 70 g), HE: housing environment, DL: deep litter, PC: *Petroselinum crispum*, MP: *Mentha piperita*, MS: *Medicago sativa*, LS: Lohmann Sandy, LW: Lohmann LSL Classic, n: number of eggs. Significant difference at $p<0.05$.

Egg-laying time affected USDA egg size distribution ($p<0.01$), with more Sm, M, L, XL, and XXL laid at 9:00 a.m. than at 12:00 noon (Table 6). Also, more eggs were laid in the morning, at 9:00 a.m., than at 12:00 noon, the percentage of which was much higher than that of 3:00 p.m. With regards to the nest box tier, there was a decreasing trend in Sm and M eggs and an increasing trend in L, XL, and XXL eggs with the upward increase in the nest box tier (Table 6; $p<0.01$). Additionally, the % of eggs laid on the upper tier was higher than that of the middle and bottom tier. Floor eggs constituted only 0.5% of the total number of eggs produced.

DISCUSSION

In this study, the biological event (i.e., increase in body weight) that comes with the aging of hens was expected across the study. Strain differences observed in HW indicate genetic influence on body weight. This would be consistent with the Lohmann breeder guide, where it has been reported that Lohmann Sandy are heavier than Lohmann LSL Classic hybrids. Similarly, genetic influence on HW has been reported by some authors (Şekeroğlu & Sarıca, 2005; Yakubu *et al.*, 2007; Ahmad *et al.,* 2019). Also, Batkowska & Brodacki (2017) observed hybrid differences regarding body weight only at 8 and 16 weeks of hen age, but no differences at 33 weeks of age.

Table 6 – Influence of egg-laying time, and nest box tier on USDA egg size distribution expressed as the number of eggs and ratio of eggs per category (%).

Parameter		USDA egg size distribution					
		Sm	M	L	XL	XXL	Total
Egg-laying time	Overall, n	1818 (3.3%)	7735 (13.8%)	31181 (55.8%)	12069 (21.6%)	3072 (5.5%)	55875 (100)
	9:00	1058 (1.9%)	3821 (6.8%)	15346 (27.5%)	7224 (12.9%)	1873 (3.4%)	29322 (52.5%)
	12:00	655 (1.2%)	3567 (6.4%)	15427 (27.6%)	4783 (8.6%)	1176 (2.1%)	25608 (45.8%)
	3:00	105 (0.2%)	347 (0.6%)	408 (0.7%)	62 (0.1%)	23 (0%)	945 (1.7%)
	x^2 (P)	1202.7 (< 0.001)					
Nest box tier	Floor	43 (0.1%)	47 (0.1%)	126 (0.2%)	43 (0.1%)	16 (0%)	275 (0.5%)
	Bottom	696 (1.2%)	3074 (5.5%)	9559 (17.1%)	3263 (5.8%)	833 (1.5%)	17425 (31.2%)
	Middle	649 (1.2%)	2610 (4.7%)	9706 (17.4%)	3554 (6.4%)	1057 (1.9%)	17576 (31.5%)
	Upper	430 (0.8%)	2004 (3.6%)	11790 (21.1%)	5209 (9.3%)	1166 (2.1%)	20599 (36.9%)
	x^2 (P)	962.5 (< 0.001)					

Abbreviations: Sm: small (42.0-49 g), M: medium (49-56 g), L: large (56-65 g), XL: extra-large (65-70 g), XXL: jumbo (≥70 g). Significant difference at $p<0.05$.

In the present study, hens in outdoor plant housing environments were heavier than deep litter hens only at 19 weeks of age, with no differences at other weeks.

Similarly, Oke *et al*. (2016) reported a significantly higher HW at 20 weeks of age for hens that accessed outdoor legume and grass pastures than deep litter hens, but no difference in HW across rearing systems at 38 and 60 weeks of age, the end of the study. These results could be attributed to the pattern of changes in the range use of hens and consequently, the additional intake of nutrients from the different plant compositions that come with the season of the year and the aging of hens. Furthermore, higher body weight in free-range than completely indoor birds has been associated with additional dietary resources such as plant species, insects, and worms, as well as sand gravel, resulting in a higher proportion of the digestive system and changes in internal organs (Obeng *et al.,* 2013; Yang *et al.,* 2014). These benefits have been related to the enhanced consumption rate of various nutrients including energy, protein, amino acids, and minerals (Obeng *et al.,* 2013), resulting in better body weights. Again, it can be speculated that at some point the improved untilization rate of various nutrients might compensate for the lost nutrients due to increased activity, which is one of the possible causes of depressed body weight in the free-range birds.

In the present study, AFE and ASM were lower and similar across the outdoor plant housing environments than for deep litter hens. This would be in agreement with Oke *et al*. (2016), who reported significantly lower AFE in hens granted access to legume pasture than for hens permitted access to grass pasture and a deep litter system. Also, other previous studies identified that free-range hens reached ASM earlier than hens housed completely indoors (Yılmaz Dikmen *et al*., 2016; Şekeroğlu *et al*., 2010), which is in line with the current results. It is worth noting that the exposure to greater intensities of UV from sunlight might be linked to the activating of gonadal development and synthesis of steroid hormones, especially oestradiol, which stimulates the development of the reproductive tract, secondary sex characteristics, and ovulation (England & Ruhnke, 2020). This could be the explanation for why the hens with outdoor access reached sexual maturity much earlier than the birds reared completely indoor.

In the current study, strain differences in AFE and ASM were observed, suggesting a genetic influence on both traits. Similarly, Şekeroğlu & Sarıca (2005) demonstrated that brown layer hybrids reached ASM earlier than the white hybrids. It is also important to note that in the present study, the ASM for both strains (LS strain, 135.08 days and LW strains, 140.38 days) were lower than the recommended (140-145 days) in the Lohmann management guide (Lohmann, 2022), which might be linked to the housing environment or region of the study.

The present study showed no differences in liveability among housing environments and between strains. However, the liveability of LS and LW strains during the rearing and egg-laying periods (99.23 vs. 97.69% and 99.23 vs. 100%, respectively) was within the recommended percentages reported in the Lohmann management guide (Lohmann, 2022), for both strains under alternative housing (98-99% and 94-95% in rearing and egg-laying period, respectively). Similarly, a lack of genetic influence on liveability was also found in some previous studies (Şekeroğlu & Sarıca, 2005; Yakubu *et al*., 2007). The results of the present study contrast with previous research (Yakubu *et al*., 2007; Alig *et al*., 2023a), where liveability differed among housing systems. However, these studies compared the free range and cage systems, which might be the cause of variation in the study findings.

This study agreed with the study by Şekeroğlu & Sarıca (2005), which did not identify differences in HHE between hens in deep litter and free-range systems. In contrast, previous studies demonstrated differences in HHE across different housing environments (Yakubu *et al*., 2007; Alig *et al*., 2023a, 2023b). It is suggested that the differences in the specific housing environments and strains that were studied could be the source of deviation among study results. The results of the current study indicated no genetic influence on HHE, which would not agree with Şekeroğlu & Sarıca (2005), who found higher HHE for brown layer hybrids than white layers. Also, studies by Alig *et al*. (2023a, 2023b) speculated genetic influence on HHE.

The present study agreed with previous studies that found significant variations in HDE among housing environments (Alig *et al*., 2023a, 2023b). In contrast, HDE has also been found to be similar between deep litter and free-range hens (Şekeroğlu & Sarıca, 2005). In the current study, significant differences were obtained for HDE among the hens that were allowed access to different outdoor plant compositions. These results suggest that different plant compositions contain specific bioactive compounds, and their intake at the required amounts may improve gut and oviduct health, consequently enhancing egg production. Indeed, Oke *et al*. (2016) identified that hens that accessed legume pastures had a better HDE than deep litter hens, whose value was higher than the hens granted access to grass pastures. In the current study, genetic influence on HDE was identified, which is not in agreement with

(Şekeroğlu & Sarıca, 2005; Rakonjac *et al*., 2021). The differences in results could be related to the studied strains and housing systems.

This study identified that MS, PC, and MS hens consumed the less feed during the egg-laying period than DL hens. It could be argued that the outdoor plants motivate the hens to spend most of their time on the range and perform other behaviours than feeding. Also, the intake of bioactive compounds from these plants could be associated with normal appetite (Buckner *et al*., 1945), ensuring an intermediate intake of plants and feed. However, this study differs from Şekeroğlu & Sarıca (2005), who reported that FI was similar between free-range and deep litter hens across the rearing and egg-laying periods. In the present study, strain differences in FI were observed, which is in line with some previous studies (Şekeroğlu & Sarıca, 2005; Küçükyılmaz *et al*., 2012), which also showed genetic influences on FI. Nonetheless, Şekeroğlu & Sarıca (2005) found strain differences in FI during the rearing period, but no differences during the egg-laying period.

The present study indicated that FCR was different among the housing environments, being lower in MS and MP than PC, whose ratio was lower than that of DL hens. Similarly, Oke *et al*. (2016) found lower FCR in hens reared with access to grass pastures than with access to legume pastures, whose value was lower than deep litter hens. Again, these results highlight that the intake of different plant compositions is related to specific bioactive compounds, which might improve gut health and boost the increase in the efficacy of feed conversion. In contrast to this study, Şekeroğlu & Sarıca (2005) reported similar FCR for hens housed with or without access to outdoor areas. Strain difference in FCR was not observed in the present study, which is not in accordance with several studies (Küçükyılmaz *et al*., 2012; Rakonjac *et al*., 2021), which reported genetic influence on the FCR of laying hens.

Results of age effects on AEW were expected and clearly reflect a well-known biological event that comes with the aging of hens. In agreement with this study, previous studies reported differences in AEW among housing environments (Alig *et al*., 2023a, 2023b). However, these later studies observed that the AEW from free-range hens was higher than that of hens that were completely indoors, which is partially contrary to the present study.

In the present study, there was a decreasing trend in average egg weight across the time of day, which might indicate that in hens that lay heavy eggs, oviposition occurs in the morning hours. Similar results have been reported by some other studies (Tůmová & Ebeid, 2005; Tůmová & Gous, 2012; Zakaria & Omar 2013; Samiullah *et al*., 2016). However, this would be inconsistent with Tumova *et al*. (2017b), who observed no effect of egg-laying time on egg weight. It can be argued that the differences in results may be due to differences in the housing system, the strains that were used, etc.

In the present study, heavier eggs were obtained from the upper tier, and lighter eggs were collected from the floor, suggesting that hens that lay heavy eggs preferred nest boxes placed 94 cm off the ground. On the other hand, floor eggs may be associated with hens that lay lighter eggs.

The age effect on egg size distribution clearly reflects the biological pattern of changes in egg weight that occur with the increase in the age of hens, which is always expected during egg-laying. This study identified variations in egg size distribution among the housing environments, which is consistent with Alig *et al*. (2023a, 2023b), who reported significant differences in egg size distribution in free-range, enriched, and colony cage systems. However, their studies showed more XL eggs from free-range hens than indoor hens, which is contrary to the present study.

In the present study, the differences identified in egg size distribution between strains suggest genetic influence on this trait, as indicated by Alig *et al*. (2023a, 2023b). In this study, egg-laying time and nest box tier effect on egg size distribution merely confirm the pattern of changes observed in egg weight across the day. Additionally, it might be linked to the significant variation in the number of eggs at each egg collection time and nest box tier.

This study agrees with several other studies that found that most eggs were laid in the morning hours (Tůmová & Ebeid, 2005; Tůmová *et al*., 2009; Tůmová & Gous, 2012; Tůmová *et al*., 2017a). Studies have demonstrated that the most eggs are laid 1 to 5 hours after the lights are turned on (Tůmová *et al*., 2009; Samiullah *et al*., 2016). In the present study, lights were turned on at 6:00 p.m. daily, with the most eggs being collected at 9:00 a.m. followed by 12:00 noon, which are respectively 3 and 6 hours after the lights were turned on.

The present study is not consistent with previous studies, which reported that hens preferred ground-level nests (Krause & Schrader, 2018; Bari *et al*., 2020). In the present study, it can be suggested that most of the individuals within the strains were characterized by high kinetic energy, resulting in better impulse during take-off, landing accuracy, and longer latency to jump (Rufener *et al*., 2020). Thus, these hens were ready and always attempted to lay on the upper next box tier. This study indirectly suggests that nest box height matters to the hens, as is the case of perch height, and most of the hens may prefer nest boxes raised at least 94 cm off the ground.

CONCLUSIONS

This study was able to determine that deep litter with access to *Medicago sativa, Petroselinum crispum, and Mentha piperita* is appropriate for optimizing the production parameters of hens, and may have the economic advantage of reducing feed costs. Access to *Medicago sativa* ranked highest in terms of overall production performance results. Furthermore, the adaptation of the two genetic strains to cage-free environments seems to not differ.

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