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The effect of cage density and meat storage period on some meat quality parameters in brown and white spent hens

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Abstract

This study was conducted to determine the effect of different cage densities and meat storage periods on some meat quality parameters in drumstick and breast meat of brown and white spent hens. The study used three different cage densities as low (5 hens *per* cage), normal (seven hens *per* cage) and high (10 hens *per* cage) including 396 hens as 198 Hy-Line Brown (HB) and 198 Isa Tinted (IT). The feeding of chickens was *ad libitum* (20 - 60 weeks). At the end of the study, a total number of 54 chickens (27 HB and 27 IT) were slaughtered. Some chemical and microbiological analyses were carried out by separating the drumstick and breast area of the slaughtered chickens. According to the results of the research, the redness and yellowness values of the breast area were higher in the ITs. The effect of cage density was significant only for yellowness in the drumstick area. The effects of storage time, lightness and yellowness in the drumstick area and lightness value in the breast area were found to be significant. Bacterial density and thiobarbituric acid reactive substances values of both breast and drumstick regions of spent hens were higher in HBs, while pH values were higher in ITs. The bacterial density in the meat was increased during the storage period. In conclusion, cage density, genotype and storage time affected the microbiological and chemical quality of spent chicken meat. With this result, it can be said that meat quality studies, which mostly focus on broilers, are also important in spent hens.

Introduction

Droughts caused by global climate change adversely affect plant and animal production, reducing the quantity and quality of the products that can be obtained from them. In addition, the gradual increase in the world population highlights the need to maximize the use of plant and animal products. As a result, research in animal production is progressing rapidly to increase productivity. Chickens are one of the leading breeds among animal protein sources because they are affordable, inexpensive and produce high quality meat.[1] Laying hens are usually culled at 80 - 90 weeks after they have completed the economic production period or they can be kept in the flock for another year for egg production using forced molting methods.[2] Hens removed from the flock are considered spent hens and are slaughtered. They are used to make production of products such as meatballs, salami,and sausages. Chicken farmers can cover a large part of the cost of replacement chickens with the income they earn from disposing of chickens using this method.[3,4]

In commercial poultry production, it is necessary to increase the density of birds in cages in order to obtain more product *per* unit area and to reduce production costs. Studies in poultry indicate that increasing the cage density has a negative effect on performance, health and welfare. The effect of cage density is generally a decrease in carcass weight and egg production, an increase in feed conversion ratio and an increase in the rate of foot inflammation.[5-10]

Studies on the effect of cage density on chicken meat quality have mostly focused on broilers.[11-14] The aim of this study was to compare the effect of cage density on some quality criteria in Hy-Line Brown (HB) and Isa Tinted (IT) spent hens.

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Materials and Methods

Animals and study design. The research was carried out in a laying hen house and in a single building. The layer house consisted of three blocks, two rows and four tiers of battery type cages. These cages had a floor slope of 7.00º. The cages were 51.00 cm high at the front, 46.00 cm high at the back, 60.00 cm deep and 62.50 cm wide. A light period of 16 hr light and 8 hr dark was applied in the hen house. A total number of 396 laying hens, 198 HB and 198 IT, were included in the study. The hens feeding was fed *ad libitum* from the pullet stage (week 20) to week 60. Three different cage densities (five hens *per* cage; 750 cm^2 *per* hen, 7 hens *per* cage; 535.71 cm^2 *per* hen, 10 hens *per* cage; 375 cm^2 *per* hen) were set up in the study. The body weights of the chickens were measured before they were placed in the cages. Hybrids with similar body weights were randomly placed in the cages. Uniformity in terms of body weight was achieved 94.00 % for white hybrids and 93.00 % for the brown hybrids. Subgroups were formed with nine replications. At the end of the study period (week 60), a total number of 54 HB and IT, one from each cage, were slaughtered. Prior to slaughter, it was ensured that all materials to be used in slaughtering were aseptic. To this end, just prior to slaughter, the knives used to slaughter the animals were washed with alcohol and passed through the flame for sterilization. The drumsticks and breasts of the slaughtered chickens were individually placed in plastic bags. The samples were then taken to the Food Hygiene and Technology Laboratory of the Veterinary Faculty of Atatürk University and stored at + 4.00 ˚C for 24 hr. On days one, four, seven and 10 the samples were taken from the stored leg and breast meat for total bacterial count, meat quality characteristics and lipid per-oxidation analysis. This study was conducted at the Poultry Unit of the Food and Livestock Research and Application Centre of Atatürk University, Erzurum. The study was approved by Atatürk University Faculty of Veterinary Medicine Ethics Committee (decision No. 2023/07; date: 08.03.2023).

Determination of total bacteria count. For microbiological analysis, 25.00 g of meat samples were weighed under aseptic conditions in a special sterile stomacher plastic bag, 225 mL of Ringer's solution (Merck, Darmstadt, Germany) was added and homogenized in the homogenizer. In this way, a 10^{-1} dilution was prepared and a serial dilution was prepared by taking 1.00 mL from the first dilution using sterile pipettes and transferring it to a tube containing 9.00 mL of sterile dilution liquid. Plate count agar medium (Merck) was used to count total *mesophilic* aerobic bacteria (TMAB). Counting was performed after incubation for 48 hr at 37.00 ˚C by seeding 1.00 mL of dilutions appropriate for the medium using the pour plate method. De Man Rogosa Sharpe agar (Merck) was used to determine the number of *Lactobacillus*. For this purpose, 1.00 mL of appropriate dilutions were seeded by the pour plate method and counted after incubation in an anaerobic environment (Anaerocult A) for 72 hr at 37.00 ˚C. To determine the number of *Lactococcus*, M17 agar (Merck) was used. Appropriate dilutions (1.00 mL) were seeded into petri dishes using the pour-plate method and counted after incubation at 37.00 ± 1.00 ˚C for 48 hr. Plate count agar medium was used for counting total *psychrophile* aerobic bacteria (TPAB). Appropriate dilutions (1.00 mL) for the medium were cultured by the cast-plate method and counted after 10 days of incubation at 7.00 ± 1.00 ˚C. For counting *Pseudomonas* bacteria, *Pseudomonas* agar was prepared and mixed with cetrimide-fucidin-cephalotin supplement (Oxoid, Basingstoke, UK). The dilutions (1.00 mL) suitable for the medium were cultured by the smear plate method and incubated at 20.00 ˚C for 48 hr. Colonies greater than 1.00 mm in diameter and catalase positive ones were counted. The bacterial counts were expressed in log colony-forming unit *per* g.[15]

Meat quality characteristics. The meat sample (10.00 g) was mixed with 100 mL of distilled water and homogenized in an Ultra Turrax Mixer (IKA T18, Germany). The pH of the prepared mixture (inolab WTW, Weilheim Germany) was measured using a digital pH meter. A portable hygrometer (4TE; Aqua LAB, Santa Clara, USA) was used to determine the water activity (a_w) value of the samples. Color analysis of the meat was performed using a Chroma Meter (Konica Minolta, Tokyo, Japan). The results were described according to the parameters L* (lightness), a* (redness) and b* (yellowness).[16]

Lipid peroxidation analysis. For the analysis of thiobarbituric acid reactive substances (TBARS) values, 2.00 g of the homogenized samples were taken and 12.00 mL of TCA (trichloroacetic acid) solution (7.50% TCA, 0.10 % ethylenediaminetetraacetic acid, 0.10% propyl gallate dissolved in 3.00 mL ethanol) (Merck) was added. The samples were then homogenized in Ultra-Turrax for 15 - 20 sec and then filtered through 1.00 Whatman filter paper. 3.00 mL of the filtrate was transferred to the test tube and 3.00 mL of thiobarbituric acid (0.02 M) solution was placed on it and homogenized and the test tubes were kept in a water bath at 100 ˚C for 40 min and then cooled in cold water for 5 min. After centrifugation (5 min at 2,000 *g*) the absorbance values were read at 530 nm in a spectrophotometer (MQX200; BioTek Instruments, Winooski, USA) and the results were expressed as µmol malonaldehyde kg^{-1}. The TBARS was measured as follows: [16]

$$TBARS = \frac{((absorbance / k\ (0.06) \times 2 / 1{,}000) \times 6.80) \times 1{,}000}{sample\ weight}$$

Statistical analysis. The experiment was designed as a completely randomized design. Two-way ANOVA was then performed using the GLM procedure and differences between groups were evaluated using Duncan's multiple comparison test using SPSS Software (version 18.0; IBM Corp., Armonk, USA). The linear model for testing the effects of treatment groups on chemical, microbiological and color parameters was as follows.

$$Yijkl = \mu + G + CD + D + (GxCDxD) + eijkl$$

where, *Yijkl* = response variable, μ = population means, *G* = genotype (HB, IT), *CD* = cage density (low, normal, high), *D* = day (1^{st}, 4^{th}, 7^{th}, 10^{th}), *GxCDxD* = genotype × cage density × day interaction, and *Eijk* = experimental error.

Results

Table 1 shows the L* values in the drumstick area which differed according to the storage periods. The L* value was the highest on day 10 and the lowest was detected on day one. It was found that the effect of cage density on the b* value of drumstick meat was significant ($p < 0.05$). The mean b* values in the low cage density (LCD), normal cage density (NCD) and high cage density (HCD) groups were found to be 3.29, 3.65 and 4.57, respectively. It was determined that the effect of the day on the b* value of drumstick meat was significant ($p < 0.001$; Table 1).

In the current study, a significant difference was found between a* values of the hybrids ($p < 0.05$; higher in IT; Table 2). In addition, a* value of the breast meat significantly differed according to the days ($p < 0.001$). The effect of genotype ($p < 0.05$) and day ($p < 0.001$) on b* value of the breast meat was found to be significant (higher in ITs; Table 2).

Total *mesophilic* aerobic bacteria count values in the drumstick meat of HB and IT laying hens were 5.02 ± 0.05 and 5.40 ± 0.05, respectively ($p < 0.001$; Table 3). In addition, cage density and the effect of storage time on TMAB in drumstick meat were also found to be important ($p < 0.001$). It was found that the effect of genotype, cage density and storage period on the number of *Coliform* group bacteria number in drumstick meat was significant ($p < 0.001$; Table 3). It was found that the number of *Coliform* bacteria was higher in the drumstick meat of white spent hens. Moreover, the number of *Coliform* group bacteria in drumstick meat was observed to be higher in dense cage density. The effect of genotype and cage density on *Micrococcus* group bacteria in the drumstick meat was found to be significant ($p < 0.01$). The number of *Micrococcus* group bacteria in the drumstick meat on day one was 3.34,on day four was 3.92, on day seven was 4.45 and on day 10 was found to be 4.90. It was found that the effect of genotype and day on the number of TPAB in drumstick meat was significant ($p < 0.001$; Table 3).

The effects of genotype ($p < 0.001$), cage density ($p < 0.001$) and day ($p < 0.05$) on the pH value of drumstick meat were found to be significant (Table 3). When a_w values in drumstick meat were examined, the effect of storage period was found to be significant ($p < 0.001$).

Table 1. Variance analysis results of color parameters (L*, a* and b*) determined from the skinless drumstick meat on different storage period in brown and white laying hens housed in different cage density (mean ± SEM).

Hybrid	CD	Days	L*	a*	b*
		1	49.06 ± 2.24^{b}	9.84 ± 1.43	–0.76 ± 1.01
		4	53.07 ± 2.24^{b}	8.42 ± 1.43	3.31 ± 1.01
	LCD	7	54.81 ± 2.24^{ab}	9.07 ± 1.43	6.99 ± 1.01
		10	55.18 ± 2.24^{a}	9.47 ± 1.43	2.53 ± 1.01
		Total	53.03	9.20	2.76^{b}
		1	54.00 ± 2.24	8.36 ± 1.43	3.32 ± 1.01
		4	52.54 ± 2.24	8.62 ± 1.43	1.73 ± 1.01
Hy-Line Brown	NCD	7	52.37 ± 2.24	9.12 ± 1.43	5.74 ± 1.01
		10	58.39 ± 2.24^{a}	6.57 ± 1.43	2.32 ± 1.01
		Total	54.32	8.16	3.27^{ab}
		1	50.14 ± 2.24	9.12 ± 1.43	0.93 ± 1.01
		4	53.36 ± 2.24	8.50 ± 1.43	3.55 ± 1.01
	HCD	7	52.66 ± 2.24	9.19 ± 1.43	6.49 ± 1.01
		10	55.27 ± 2.24	11.31 ± 1.43	6.19 ± 1.01
		Total	52.85	9.53	4.28^{a}
		1	53.66 ± 2.24	9.77 ± 1.43	2.59 ± 1.01
		4	50.21 ± 2.24	9.24 ± 1.43	2.23 ± 1.01
	LCD	7	55.97 ± 2.24	9.14 ± 1.43	5.98 ± 1.01
		10	48.13 ± 2.24	8.15 ± 1.43	4.48 ± 1.01
		Total	51.99	9.07	3.81^{b}
		1	49.92 ± 2.24	9.59 ± 1.43	4.11 ± 1.01
		4	51.18 ± 2.24	9.13 ± 1.43	3.67 ± 1.01
Isa Tinted	NCD	7	55.39 ± 2.24	8.61 ± 1.43	6.39 ± 1.01
		10	62.93 ± 2.24	7.94 ± 1.43	1.92 ± 1.01
		Total	54.85	8.81	4.02^{ab}
		1	52.74 ± 2.24	8.53 ± 1.43	3.70 ± 1.01
		4	56.91 ± 2.24	9.30 ± 1.43	4.84 ± 1.01
	HCD	7	53.38 ± 2.24	9.19 ± 1.43	7.13 ± 1.01
		10	54.92 ± 2.24	7.58 ± 1.43	3.72 ± 1.01
		Total	54.48	8.64	4.84^{a}
		1	52.10^{b}	9.20	2.14^{c}
	Total	4	52.76^{b}	8.86	3.22^{bc}
		7	54.91^{ab}	9.05	6.45^{a}
		10	55.32^{a}	8.50	3.52^{b}
***p*-values**					
Hybrid			0.68	0.84	0.06
CD			0.18	0.60	0.04
Day			0.01	0.85	< 0.01
Hybrid × CD			0.49	0.56	0.89
Hybrid × Day			0.42	0.69	0.06
CD × Hybrid			0.01	0.56	0.89
Hybrid × CD × Day			0.07	0.86	0.10

Hy-Line Brown: Brown laying hen; Isa Tinted: White laying hen; CD: Cage density; L*: Relative lightness; a*: Relative redness; b*: Relative yellowness; LCD: Low cage density; NCD: normal cage density; HCD: High cage density;

^{abc} Differences between means with different superscript letters in the same column are significant ($p < 0.05$).

It was found that the effect of genotype, cage density and day on TBARS values in drumstick meat was significant ($p < 0.001$), (higher in ITs and on day 10th).

Table 4 shows that the effect of cage density on the TMAB in the breast area was significant ($p < 0.001$). The TMAB in the breast meat of the spent hens in the LCD group was lower than in the other two groups. It was found that the effect of genotype and cage density on the number of *Coliforms* bacteria found in breast meat was significant ($p < 0.001$).

Table 2. Variance analysis results of color parameters (L*, a* and b*) determined from the skinless breast meat on different storage period in brown and white laying hens housed in different cage density (mean ± SEM).

Hybrid	CD	Days	L*	a*	b*
Hy-Line Brown	LCD	1	54.65 ± 1.58	2.28 ± 0.78	2.85 ± 0.80
		4	52.73 ± 1.58	2.39 ± 0.78	4.03 ± 0.80
		7	51.84 ± 1.58	8.92 ± 0.78	6.94 ± 0.80
		10	49.58 ± 1.58	5.45 ± 0.78	5.54 ± 0.80
	NCD	1	54.82 ± 1.58	3.49 ± 0.78	3.42± 0.80
		4	51.46 ± 1.58	4.66 ± 0.78	1.27 ± 0.80
		7	56.72 ± 1.58	10.44 ± 0.78	7.04 ± 0.80
		10	56.41 ± 1.58	2.47 ± 0.78	3.58 ± 0.80
	HCD	1	53.39 ± 1.58	2.35 ± 0.78	3.99 ± 0.80
		4	51.90 ± 1.58	2.64 ± 0.78	2.59 ± 0.80
		7	54.06 ± 1.58	10.08 ± 0.78	7.36± 0.80
		10	56.09 ± 1.58	2.30 ± 0.78	5.19 ± 0.80
Isa Tinted	LCD	1	52.35 ± 1.58	3.89 ± 0.78	1.86 ± 0.80
		4	48.81 ± 1.58	6.18 ± 0.78	2.00 ± 0.80
		7	55.96 ± 1.58	9.87 ± 0.78	7.06 ± 0.80
		10	52.33 ± 1.58	3.74 ± 0.78	7.65 ± 0.80
	NCD	1	51.75 ± 1.58	4.11 ± 0.78	5.73 ± 0.80
		4	53.63 ± 1.58	4.28 ± 0.78	1.52 ± 0.80
		7	49.22 ± 1.58	9.03 ± 0.78	9.09 ± 0.80
		10	52.21 ± 1.58	2.95 ± 0.78	3.78 ± 0.80
	HCD	1	51.97 ± 1.58	5.66 ± 0.78	2.35 ± 0.80
		4	52.59 ± 1.58	4.38 ± 0.78	7.66 ± 0.80
		7	53.94 ± 1.58	8.69 ± 0.78	7.41 ± 0.80
		10	53.73 ± 1.58	2.34 ± 0.78	6.20 ± 0.80
	Total	1	53.16	3.62[b]	3.36[c]
		4	51.85	4.09[b]	3.17[c]
		7	53.62	9.51[a]	7.48[a]
		10	53.39	3.20[b]	5.32[b]
***p*-values**					
Hybrid			0.06	0.05	0.03
CD			0.28	0.38	0.07
Day			0.22	< 0.01	0.01
Hybrid × CD			0.11	0.19	0.15
Hybrid × Day			0.78	< 0.01	0.52
CD × Hybrid			0.34	0.19	0.15
Hybrid × CD × Day			< 0.01	0.07	< 0.01

Hy-Line Brown: Brown laying hen; Isa Tinted: White laying hen; CD: Cage density; L*: Relative lightness; a*: Relative redness; b*: Relative yellowness; LCD: Low cage density; NCD: Normal cage density; HCD: High cage density;

[abc] Differences between means with different superscript letters in the same column are significant ($p < 0.05$).

The mean number of *Coliform* bacteria in HB laying hens was 3.52 ± 0.04. This value was found to be 3.61 ± 0.04 in IT spent hens. It was found that the effect of genotype and cage density on the number of *Micrococcus* group bacteria in breast meat was significant ($p < 0.001$). It was found that the effect of genotype, cage density and day on the number of TPAB group bacteria found in breast meat was significant ($p < 0.001$). According to this result, the number of TPAB in the LCD and NCD groups was similar and the number of TPAB group bacteria in the HCD group was found to be higher than in the other groups. Table 4 shows that the effect of genotype on the pH value of breast meat was significant ($p < 0.05$). It was found that the pH values of the breast meat of ITs were higher. It was found that the effect of the storage period on a_w values in breast meats was significant ($p < 0.001$). It was determined that the effect of genotype and storage period on TBARS values in breast meat was significant ($p < 0.001$).

Discussion

As shown in Table 1, the effect of hybrid on the L*, a* and b* values of drumstick meat was not significant. In another study, the effect of hybrid on L* and a* values of drumstick meat were found to be significant.[12,17] L*, a* and b* values in drumstick meat of crossbred laying hen, broiler and laying hen were examined and it was determined that the difference between hybrid was significant.[18] Although this result contradicted the result of the present study, it was in agreement with the previous study. In the present study, it was observed that the b* value was increased with increasing cage density. In another study it was stated that the effect of cage density on L*, a* and b* values of drumstick meat was insignificant.[19] In the current study, the effect of storage period on the L* and b* values of drumstick meat were significant. The more increased was the L* value, the longer was the storage period. The b* value was the highest on the 7th day. In another study, it was stated that the effect of storage period (0, 3, 7, 14 and 21) on meat color was insignificant.[20] In another study, the effect of storage period (0, 3, 7, 14 and 21 days) on meat color was found not to be significant.[18] In another study, the effect of storage period on L*, a* and b* values in drumstick meat was found to be significant.[15]

As shown in Table 1, the effectiveness of the hybrid on the L*, a* and b* values of breast meat was examined. The a* and b* values of the breast meat were higher in white spent hens compared to brown spent hens. In another study, only the effect of hybrid on it was found, indicating that the effect of genotype on b* values was important.[17] In one study, L*, a* and b* values in the breast meat of broiler and laying hens were examined and the difference between genotypes was found to be significant.[18] Contrary

Table 3. Variance analysis results of the effects of storage period, genotype and cage density on microbiological activity (log colony-forming unit *per* g), pH, a_w and TBARS (mg kg^{-1}) values in skinless drumstick meat (mean ± SEM).

Days	Hybrid	CD	TMAB	Coliform	*Micrococcus-Staphylococcus*	TPAB	pH	a_w	TBARS
1	Hy-Line Brown	LCD	3.69 ± 0.16	2.21 ± 0.13	3.49 ± 0.18	2.28 ± 0.33	6.15 ± 0.04	0.99 ± 0.00	4.82 ± 0.24
		NCD	3.63 ± 0.16	2.94 ± 0.13	2.65 ± 0.18	2.64 ± 0.33	6.02 ± 0.04	0.99 ± 0.00	5.68 ± 0.24
		HCD	3.82 ± 0.16	3.12 ± 0.13	3.27 ± 0.18	3.30 ± 0.33	6.19 ± 0.04	0.99 ± 0.00	5.31 ± 0.24
	Isa Tinted	LCD	4.85 ± 0.16	3.38 ± 0.13	3.79 ± 0.18	3.66 ± 0.33	6.10 ± 0.04	0.99 ± 0.00	3.86 ± 0.24
		NCD	3.06 ± 0.16	2.98 ± 0.13	3.58 ± 0.18	3.37 ± 0.33	6.18 ± 0.04	0.99 ± 0.00	4.59 ± 0.24
		HCD	4.39 ± 0.16	2.33 ± 0.13	3.27 ± 0.18	3.32 ± 0.33	6.28 ± 0.04	0.99 ± 0.00	4.92 ± 0.24
4	Hy-Line Brown	LCD	4.23 ± 0.16	2.61 ± 0.13	4.04 ± 0.18	3.54 ± 0.33	6.15 ± 0.04	0.99 ± 0.00	5.49 ± 0.24
		NCD	4.63 ± 0.16	3.63 ± 0.13	3.00 ± 0.18	4.09 ± 0.33	6.13 ± 0.04	0.99 ± 0.00	6.23 ± 0.24
		HCD	4.54 ± 0.16	4.48 ± 0.13	3.65 ± 0.18	4.55 ± 0.33	6.10 ± 0.04	0.99 ± 0.00	5.87 ± 0.24
	Isa Tinted	LCD	6.26 ± 0.16	4.18 ± 0.13	4.37 ± 0.18	4.74 ± 0.33	6.26 ± 0.04	0.99 ± 0.00	3.94 ± 0.24
		NCD	4.48 ± 0.16	3.87 ± 0.13	4.22 ± 0.18	4.49 ± 0.33	6.21 ± 0.04	0.99 ± 0.00	4.81 ± 0.24
		HCD	4.96 ± 0.16	3.35 ± 0.13	4.26 ± 0.18	4.22 ± 0.33	6.23 ± 0.04	0.99 ± 0.00	5.05 ± 0.24
7	Hy-Line Brown	LCD	5.66 ± 0.16	3.22 ± 0.13	4.86 ± 0.18	5.34 ± 0.33	6.05 ± 0.04	0.99 ± 0.00	5.44 ± 0.24
		NCD	5.29 ± 0.16	4.04 ± 0.13	3.49 ± 0.18	5.00 ± 0.33	6.10 ± 0.04	0.99 ± 0.00	6.77 ± 0.24
		HCD	5.61 ± 0.16	4.89 ± 0.13	4.10 ± 0.18	5.17 ± 0.33	6.03 ± 0.04	0.99 ± 0.00	6.39 ± 0.24
	Isa Tinted	LCD	6.44 ± 0.16	4.77 ± 0.13	5.18 ± 0.18	5.75 ± 0.33	6.53 ± 0.04	0.99 ± 0.00	4.72 ± 0.24
		NCD	4.93 ± 0.16	3.98 ± 0.13	4.75 ± 0.18	5.67 ± 0.33	6.23 ± 0.04	0.99 ± 0.00	5.11 ± 0.24
		HCD	5.30 ± 0.16	3.92 ± 0.13	4.29 ± 0.18	5.26 ± 0.33	6.19 ± 0.04	0.99 ± 0.00	5.81 ± 0.24
10	Hy-Line Brown	LCD	6.76 ± 0.16	4.19 ± 0.13	4.98 ± 0.18	6.72 ± 0.33	6.18 ± 0.04	0.99 ± 0.00	6.16 ± 0.24
		NCD	6.04 ± 0.16	4.23 ± 0.13	4.17 ± 0.18	6.95 ± 0.33	6.17 ± 0.04	0.99 ± 0.00	6.96 ± 0.24
		HCD	6.34 ± 0.16	5.25 ± 0.13	4.40 ± 0.18	6.72 ± 0.33	6.05 ± 0.04	0.99 ± 0.00	7.25 ± 0.24
	Isa Tinted	LCD	7.55 ± 0.16	5.07 ± 0.13	5.91 ± 0.18	6.69 ± 0.33	6.58 ± 0.04	0.99 ± 0.00	5.47 ± 0.24
		NCD	6.00 ± 0.16	4.06 ± 0.13	4.99 ± 0.18	8.55 ± 0.33	6.25 ± 0.04	0.99 ± 0.00	6.51 ± 0.24
		HCD	6.53 ± 0.16	4.69 ± 0.13	4.97 ± 0.18	7.41 ± 0.33	6.15 ±0.04	0.99 ± 0.00	6.52 ± 0.24
***p*-values**									
Hybrid			< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.19	< 0.01
CD			< 0.01	< 0.01	< 0.01	0.31	< 0.01	0.56	< 0.01
Day			< 0.01	< 0.01	< 0.01	< 0.01	0.04	< 0.01	< 0.01
Hybrid × CD			< 0.01	< 0.01	< 0.01	0.07	< 0.01	0.59	0.11
Hybrid × Day			< 0.01	0.64	0.32	0.69	< 0.01	0.21	0.15
CD × Hybrid			< 0.01	< 0.01	< 0.01	0.08	< 0.01	0.59	0.11
Hybrid × CD × Day			0.05	0.03	0.35	0.13	0.01	0.41	0.40

Hy-Line Brown: Brown laying hen; Isa Tinted: White laying hen; CD: Cage density; TMAB: Total number of bacteria; TPAB: Number of psychrophilic bacteria; LCD: Low cage density; NCD: Normal cage density; HCD: High cage density; a_w: Water activity; TBARS: Thiobarbituric acid-reactive substances.

to the present and previous studies, it was stated in another study that the effect of hybrid on L*, a* and b* values in of breast meat was insignificant.[12,21] In the present study, it was determined that the effect of cage density on L*, a* and b* values in of breast meat was found to be insignificant. Other studies, consistent with the present study, found that the effect of cage density on L*, a* and b* values of breast meat was insignificant.[11,19,21] When the effect of storage period on L*, a* and b* values of breast meat was investigated, it was determined that the effect on a* and b* values was significant. In another study similar study, the effect of storage period on L* and a* values of breast meat were significant.[15]

As shown in Table 3 and 4, it was observed that the bacterial density (*Coliforms, Micrococci- Staphylococci,* and TPAB bacteria) was higher in white spent hens compared to brown spent hens. In a study on this subject, it was found that the microbiological contamination in the meat of brown spent hens was better.[22]

In addition, in the current study, it was found that the effect of cage density on the number of TMAB, *Coliforms, Micrococcus-Staphylococcus* and TPAB bacteria in the meat of drumsticks was significant. Another study indicated that the effect of cage density on TMAB in meat was insignificant tha was not in agreement with the current study.[23] The other study found that the effect of storage period on the TMAB, *Coliform, Micrococcus-Staphylococcus* and TPAB counts bacteria numbers in drumstick meat was significant. In general, it was observed that the bacterial density increased with the storage period of the meat. In another study, it was found that the effect of storage period on the TMAB and TPAB bacteria counts in drumstick meat was important.[15,24] Others stated that the number of aerobic *mesophilic* and total *Coliform* bacteria was increased at the end of the storage period.[25] It was reported that the number of *Enterobacteriacea, Micrococcus* and TMAB group in quail meat was increased with increasing storage time.[16] The effect of hybrid on pH and TBARS in leg meat was found to be significant. While the pH was value was higher in white spent hens, TBARS

Table 4. Variance analysis results of the effects of storage period, hybrid and cage density on microbiological activity (log colony-forming unit *per* g), pH, a_w and TBARS (mg kg^{-1}) values in skinless breast meat (mean ± SEM).

Days	Hybrid	CD	TMAB	Coliform	*Micrococcus-Staphylococcus*	TPAB	pH	a_w	TBARS
1	Hy-Line Brown	LCD	3.30 ± 0.21	3.98 ± 0.13	3.16 ± 0.13	2.78 ± 0.18	5.88 ± 0.04	0.99 ± 0.00	4.67 ± 0.29
		NCD	3.25 ± 0.21	2.18 ± 0.13	3.28 ± 0.13	2.23 ± 0.18	5.78 ± 0.04	0.99 ± 0.00	4.81 ± 0.29
		HCD	4.00 ± 0.21	2.06 ± 0.13	3.07 ± 0.13	2.91 ± 0.18	5.70 ± 0.04	0.99 ± 0.00	5.14 ± 0.29
	Isa Tinted	LCD	3.02 ± 0.21	2.26 ± 0.13	3.33 ± 0.13	3.89 ± 0.18	5.95 ± 0.04	0.99 ± 0.00	5.42 ± 0.29
		NCD	4.27 ± 0.21	2.80 ± 0.13	3.17 ± 0.13	3.52 ± 0.18	6.00 ± 0.04	0.99 ± 0.00	5.31 ± 0.29
		HCD	3.65 ± 0.21	2.28 ± 0.13	2.92 ± 0.13	3.27 ± 0.18	5.89 ± 0.04	0.99 ± 0.00	4.53 ± 0.29
4	Hy-Line Brown	LCD	4.61 ± 0.21	4.25 ± 0.13	3.47 ± 0.13	3.59 ± 0.18	5.87 ± 0.04	0.99 ± 0.00	5.83 ± 0.29
		NCD	4.34 ± 0.21	3.25 ± 0.13	3.91 ± 0.13	3.50 ± 0.18	5.93 ± 0.04	0.99 ± 0.00	5.63 ± 0.29
		HCD	5.43 ± 0.21	2.98 ± 0.13	3.71 ± 0.13	3.35 ± 0.18	5.78 ± 0.04	0.99 ± 0.00	5.81 ± 0.29
	Isa Tinted	LCD	4.31 ± 0.21	3.45 ± 0.13	3.88 ± 0.13	4.70 ± 0.18	5.97 ± 0.04	0.99 ± 0.00	5.45 ± 0.29
		NCD	5.18 ± 0.21	3.23 ± 0.13	4.16 ± 0.13	4.75 ± 0.18	5.97 ± 0.04	0.99 ± 0.00	5.38 ± 0.29
		HCD	4.72 ± 0.21	3.29 ± 0.13	3.29 ± 0.13	4.53 ± 0.18	5.89 ± 0.04	0.99 ± 0.00	4.96 ± 0.29
7	Hy-Line Brown	LCD	5.23 ± 0.21	4.42 ± 0.13	3.84 ± 0.13	4.60 ± 0.18	5.84 ± 0.04	0.99 ± 0.00	6.66 ± 0.29
		NCD	5.37 ± 0.21	3.61 ± 0.13	4.23 ± 0.13	4.79 ± 0.18	5.89 ± 0.04	0.99 ± 0.00	6.44 ± 0.29
		HCD	6.23 ± 0.21	3.18 ± 0.13	4.09 ± 0.13	4.20 ± 0.18	5.87 ± 0.04	0.99 ± 0.00	6.76 ± 0.29
	Isa Tinted	LCD	5.32 ± 0.21	4.26 ± 0.13	4.17 ± 0.13	5.77 ± 0.18	5.97 ± 0.04	0.99 ± 0.00	5.57 ± 0.29
		NCD	6.27 ± 0.21	3.93 ± 0.13	4.75 ± 0.13	5.81 ± 0.18	5.82 ± 0.04	0.99 ± 0.00	6.08 ± 0.29
		HCD	5.43 ± 0.21	3.81 ± 0.13	4.09 ± 0.13	5.77 ± 0.18	5.84 ± 0.04	0.99 ± 0.00	5.06 ± 0.29
10	Hy-Line Brown	LCD	6.04 ± 0.21	4.53 ± 0.13	4.29 ± 0.13	6.73 ± 0.18	5.83 ± 0.04	0.99 ± 0.00	7.06 ± 0.29
		NCD	6.38 ± 0.21	3.88 ± 0.13	4.66 ± 0.13	7.09 ± 0.18	5.85 ± 0.04	0.99 ± 0.00	7.05 ± 0.29
		HCD	6.82 ± 0.21	3.87 ± 0.13	4.94 ± 0.13	6.04 ± 0.18	6.01 ± 0.04	0.99 ± 0.00	7.28 ± 0.29
	Isa Tinted	LCD	7.02 ± 0.21	4.65 ± 0.13	4.53 ± 0.13	7.72 ± 0.18	5.88 ± 0.04	0.99 ± 0.00	5.78 ± 0.29
		NCD	7.38 ± 0.21	4.57 ± 0.13	4.49 ± 0.13	7.71 ± 0.18	5.79 ± 0.04	0.99 ± 0.00	6.17 ± 0.29
		HCD	6.38 ± 0.21	4.79 ± 0.13	4.61 ± 0.13	7.22 ± 0.18	5.84 ± 0.04	0.99 ± 0.00	6.18 ± 0.29
***p*-values**									
Hybrid			0.07	< 0.01	< 0.01	< 0.01	0.01	0.29	< 0.01
CD			< 0.01	< 0.01	< 0.01	< 0.01	0.13	0.34	0.61
Day			0.07	0.09	0.26	< 0.01	0.52	< 0.01	< 0.01
Hybrid × CD			< 0.01	< 0.01	< 0.01	0.96	0.34	0.02	0.02
Hybrid × Day			0.12	< 0.01	0.10	0.29	< 0.01	0.52	0.01
CD × Hybrid			< 0.01	< 0.01	< 0.01	0.96	0.34	0.02	0.02
Hybrid × CD × Day			0.28	< 0.01	0.44	0.10	0.07	< 0.01	0.54

Hy-Line Brown: Brown laying hen; Isa Tinted: White laying hen; CD: Cage density; TMAB: Total number of bacteria; TPAB: Number of psychrophilic bacteria; LCD: Low cage density; NCD: Normal cage density; HCD: High cage density; a_w: Water activity; TBARS: Thiobarbituric acid-reactive substances.

value was higher in brown spent hens. The finding that hybrids had no effect on a_w value in other studies supported the present study.[26,27] In a similar study, the effect of genotype on the pH value of the drumstick area was found to be important.[12,17] It was found that the pH value of drumstick meat in brown spent hens was higher than in white spent hens.[3] In contrary to the current study,[27] it was found that the effect of genotype on the TBARS in value of drumstick meat was insignificant. In the current study, pH and TBARS values in drumstick meat were influenced by cage density. The pH value was lower in the HCD group compared to the other groups and the TBARS value was lower in the LCD group than in the other groups. Another study found that the effect of cage density on the pH value of both breast and drumstick meat of broilers was not significant.[28,29] Others found that meat quality was not deteriorated with increasing cage density and that the effect of storage period on pH, a_w and TBARS levels in drumstick meat was significant.[30] Another study found that storage period affected the meat quality of laying hens with an increase in pH from day zero to day 14.[4] The effect of storage period on the a_w value of drumstick meat has been reported to be important and supported the result of the current study.[31] In another study, the TBARS value was not much affected by the storage period of meat between day zero and day 14. This value was increased on the 21st day.[4] In another study, it was stated that the storage period had a direct effect on the TBARS value in the drumstick due to its effects on the lipid peroxidation in the meat.[15] In another study, it was found that the pH value of drumstick meat was increased on the 1st, 3rd, 5th, 7th, 9th, 11th, 13th and 15th day of storage, remained stable on the 7th day and increased again after the 10th day.[24] Genc *et al*., stated that the pH value may vary depending on the breed, the season and the stress experienced by the animal.[32]

In the present study, the effect of hybrid on the number of *Coliform, Micrococcus- Staphylococcus* and TPAB bacteria in breast meat was higher in white spent hens. In addition, it was found that the effect of cage density on the

TMAB, *Coliform, Micrococcus- Staphylococcus*, and TPAB groups bacteria in breast meat was significant. The result of another study was similar to the result of the present study and it was stated that the effect of cage density on bacterial contamination in breast meat (TPAB, *E. coli*, and *Staphylococcus aureus*) was significant, and the contamination rate was increased with increase in cage density.[33] It was found that the breast meat of Novogen White, Atak-S and Isa Brown chickens was different in terms of TAMB, TPAB, *Enterobacteriaceae*, *Coliforms* and *S. aureus* bacteria density at LCD.[21] It was found that the effect of the storage period on the number of TPAB count in breast meat was significant and that this value was increased with increase in storage time. In another study, it was found that the effect of storage period on TMAB and TPAB bacteria counts in breast meat was significant.[21] It was stated in another study that the number of *pseudomonas* and *psychrophilic* bacteria was increased with increase in the storage period of breast meat.[34] At 8, 10 and 12 storage periods, the number of *S. aureus* in breast meat was found to increase linearly.[26] Contrary to current and previous studies, the effect of storage time on TMAB in breast meat was found to be insignificant.[20] The effect of hybrid on pH and TBARS in breast meat was found to be important. It was observed that the pH value was higher in the breast meat of white spent hens and the TBARS value was higher in brown spent hens. Other studies similar to the results of the current study, found that the effect of the hybrid on the pH of the breast area was important[3,12] and that the pH of the breast meat of white spent hens was higher.[3] Contrary to the current and previous study, in another study, it was found that the effect of hybrid on the pH value of the breast area was insignificant.[17,21] Another study found that the effect of genotype on the TBARS value of breast meat was insignificant, which contradicted the current study.[15] In the current study, the effect of genotype on the a_w of breast meat was found to be insignificant, and another study supported this finding.[27]

The effect of cage density on pH, a_w and TBARS in breast meat was found to be insignificant. In other studies, with comparable results, the effect of cage density on the pH value of the breast area was found to be insignificant,[14,21] which contradicted the results of the current and previous study. It was stated that the cage density was effective on breast meat, and the pH value was associated with cage density.[19] In other studies, the insignificant effect of cage density on the a_w value of breast meat agreed with the present study.[11,21,33] Contrary to the results of the current study, in another study, it was found that the effect of cage density on the TBARS in breast meat was significant.[11,19,21,35] It was observed that the TBARS value was increased with increasing frequency.[19, 21] In the present study, the effect of storage period on a_w and TBARS values in breast meat was found to be significant. In a study in which the storage periods of breast meats were adjusted to 1, 3, 5, 7 and 9 days, it was determined that the a_w of the meat was higher on the 1st, 3rd, 5th and 7th days compared to the 9th day.[20] Another study, consistent with the results of this study, found that storage period had a direct effect on TBARS in breast meat.[15] It was found that TBARS in breast meat was increased with increase in storage period.[20]

It was found that spent hens were influenced by microbiological and chemical structure, cage density, hybrid and storage periods. In general, the bacterial density in the drumstick and breast area was higher in white chickens. In addition, the bacterial density in meat was increased with the prolongation of the storage period. The a* and b* values, which are among the color parameters of meat, were higher in white chickens. Although the pH values of the drumstick and breast area were higher in white spent hens, TBARS values were higher in brown spent hens.

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Conflicts of interest

The authors declare that they have no conflicts of interest with regard to the publication of this paper.

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