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RESEARCH PAPER

The Effect of Different Layer Genotypes Raised in The Free-range System on Egg Quality Storage at Different Temperatures

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Abstract

The aim of this study was to investigate the effect of stored eggs obtained from different layer genotypes raised in a Free-range system on egg quality at different storage temperatures. Lohmann Brown, Lohmann Sandy, and ATAK-S were used as layer genotypes in the study. The study was performed in a 3x2 factorial design with three genotypes and two storage temperatures. A total of 300 table eggs were used in the study. Egg quality analysis was carried out on 100 eggs from each genotype. Eggs were stored in refrigerator (4±2°C) and at room temperature (22±2°C) for 28 days. Egg quality was determined in 10 eggs from each group after 0, 7, 14, 21, and 28 days of storage, and the effects of temperature, genotype, and temperature x genotype interaction were determined. In the study, the effects of genotype and storage temperature on egg weight loss, Haugh unit, yolk index, and albumen pH were determined to be significant ($P<0.05$). However, the interaction effects of storage temperature x genotype were not statistically significant for any period of storage on egg quality characteristics such as Haugh unit and albumen pH. As a result, it was determined that eggs stored at refrigerator temperature during the research, depending on the storage conditions, preserved their quality characteristics better than those stored at room temperature. The study concluded that the eggs of the ATAK-S genotype had a lower shelf life compared to those of the Lohmann Sandy and Lohmann Brown genotypes.

Introduction

Eggs have been utilized as a dietary source for humans since ancient times, owing to their high biological value (Doğan 2008). Because it contains proteins and other nutrients in the egg structure and retains all of its biological value, it is used as an indicator for the quality of vegetable proteins (Durmuş 2014). Egg production is mostly done in conventional cages in the world as well as in our country. However, with the results obtained from studies conducted in recent years, it has been determined that chickens raised in traditional cages cannot fully meet their physiological needs and behavioural activities. (Bozkurt 2009). Since 2012, countries in the European Union have prohibited the use of conventional cage systems to produce eggs, and alternative methods have been recommended (Directive 1999). The free-range system is one of these producing methods. The need to determine the layer genotypes that will be used in the free-range system's egg production is increasing. Researchers have undertaken a lot of studies for this specific reason (Türker et al., 2017).

In our country, ATAK-S, Lohman Brown, Nick Brown layer hybrids are generally used in the free-range system. The Lohman Brown breed is a hybrid of foreign origin, established in Turkey through the utilization of the free-range farming. The Ankara Poultry Institute. developed the ATAK-S layer hybrid, which produces brown-shelled eggs (Goger et al., 2016). It is preferred in free-range systems and small family breeding production models in Turkey (Tutkun et al., 2018). In recent years, genotypes that produce eggs with pink-cream colored shells, have lower feed consumption than brown layers, and are commonly called tinted (Lohmann Sandy, Lohmann Silver, Hy-Line Pink, Hy-Line Sonia, H&N Coral) have also begun to be used.

It has been stated that the quality of the egg is at its highest as soon as it is laid. Egg quality may deteriorate depending on storage conditions. Depending on storage conditions, egg weight loss increases (Sert et al., 2011; Akpinar et al., 2015), Haugh unit and yolk index decreases (Baylan et al., 2011; Maman and Yildirim 2022; Parmak and Aygün 2023) and albumen pH increases (Aygun and Sert 2013; Maman and Yildirim 2022; Sariyel et al., 2022). Since no study on these genotypes grown in a free-range system was found in our literature research, this study was conducted to reveal the advantages or disadvantages of the egg quality characteristics of ATAK-S, our local layer genotype, compared to foreign layer hybrids.

Materials and Methods

The research was conducted in the Department of Animal Science, Faculty of Agriculture, Selcuk University. A total of 300 eggs (10 eggs x 5 periods x 3 genotypes x 2 storage temperatures= 300 eggs) obtained from 34-week-old Lohmann Brown, ATAK-S and Lohmann Sandy layer genotypes reared in free-range system houses were used. Collected eggs were stored in storage cabinets at average room temperature (22±2°C) and refrigerator (4±2°C) for 28 days. Egg quality analyses were performed at the beginning of storage, on the 7th, 14th, 21st, and 28th days of storage. Egg weight loss, specific gravity, albumen height, Haugh unit, yolk index and albumen pH were determined as egg quality analysis. The weights of the eggs were weighed and recorded before storage. Egg weights were determined with a digital scale with a sensitivity of 0.01 g, and egg weight loss was determined with the formula below.

Egg weight loss (%) = [Before storage egg weight(g) – Period egg weight (g)] / Before storage egg weight (g) x 100.

Egg specific gravity was determined according to the Archimedes principle (Wells 1968). Egg albumen height was determined with a height gauge after the egg was broken on a flat glass surface. After determining the albumen height and egg weight, the following formula was used to calculate the Haugh unit (Haugh 1937).

Haugh Unit = 100 log (H + 7.57 – 1.7 $W^{0.37}$) where H: Albumen height (mm) W: Egg weight (g).

The egg white and yolk were separated, and the yolk was placed on a flat glass surface. The yolk index was calculated according to Funk (1948) by measuring the yolk height with a digital height gauge and the yolk diameter with a digital micrometer. The albumen pH value was determined by separating the egg albumen from the yolk, mixing the thin and thick layer of the egg albumen thoroughly, and then measuring with a pH meter. The research was conducted using a randomized plots factorial design (3x2), including three layer genotypes (Lohmann Brown, Lohmann Sandy, and ATAK-S) and two storage conditions (room temperature and refrigeration). The statistical software tool MINITAB 16 was utilized to perform the analyses, while the Tukey multiple comparison test was employed to compare the groups. As a result of statistical analysis, $P<0.05$ value was considered statistically significant.

Results

Egg Weight Loss

The effect of storage temperature, genotype, and storage x genotype interaction on egg weight loss is given in Table 1. The interaction effects of storage temperature x genotype on egg weight loss were found to be statistically significant at the before storage egg weights and on the 28th day of storage ($P<0.05$). Before storage, the highest egg weight was found in the eggs of the Lohmann Sandy genotype at 4 °C (60.27 g), and the lowest egg weight was determined in the eggs of the ATAK-S genotype (48.46 g) at 22 °C. On the 28th day of storage, the highest egg weight loss was found in eggs of the ATAK-S genotype (5.16%) stored at 23 °C, and the lowest egg weight loss was found in eggs of the Lohmann Sandy genotype (1.56%) stored at 4 °C. The effects of different storage temperatures on egg weight loss were found to be statistically significant in all periods of storage except for the before storage egg weight ($P<0.05$). In general, egg weight loss was found to be lower in eggs stored at 4 °C than in eggs stored at 23 °C ($P<0.05$). On the 28th day of storage, the weight loss in eggs stored at 4 °C was 1.67%, while the weight loss in eggs stored at 23 °C was 4.47% ($P<0.05$). The effects of genotype on egg weight loss were found to be statistically significant on before storage egg weights on the fourteenth and twenty-eighth days of storage ($P<0.05$). The before storage egg weights obtained from the ATAK-S genotype were lower than the egg weights of the other genotypes ($P<0.05$). However, the difference between the egg weights of the Lohmann Brown and Lohmann Sandy genotypes in terms of before storage egg weight was found to be statistically insignificant. On the fourteenth (14) day of storage, the egg weight loss of the Lohmann Brown genotype (1.61%) was higher than the egg weight loss (1.34%) of the Lohmann Sandy genotype ($P<0.05$), the difference between the egg weight loss of the ATAK-S genotype and the egg weight loss (1.60%) was statistically insignificant. Similarly, egg weight loss in the ATAK-S genotype was found to be higher than that of the Lohmann Sandy genotype ($P<0.05$). On the 28th day of storage, egg weight loss of the ATAK-S genotype (3.50%) was higher than that of the Lohmann Brown (2.89%) and Lohmann Sandy genotypes (2.82%) ($P<0.05$). However, the difference in egg weight loss between Lohmann Brown and Lohmann Sandy genotypes was found to be statistically insignificant.

Table 1. Effect of storage temperature, genotype and storage x genotype interaction on egg weight loss

Treatment		Before storage egg weight (g)	Egg weight loss (%)			
			7 d	14 d	21 d	28 d
Storage temperature (°C)	23	54.38	1.33[a]	2.31[a]	3.17[a]	4.47[a]
	4	55.82	0.27[b]	0.72[b]	1.17[b]	1.67[b]
	SEM	0.636	0.036	0.051	0.054	0.074
	P-value	0.114	0.000	0.000	0.000	0.000
Genotype	LS	58.80[a]	0.75	1.34[b]	2.05	2.82[b]
	A	50.04[b]	0.83	1.60[a]	2.20	3.50[a]
	LB	56.46[a]	0.82	1.61[a]	2.25	2.89[b]
	SEM	0.779	0.044	0.063	0.066	0.090
	P-value	0.000	0.336	0.005	0.072	0.000
Storage temperature (°C) x Genotype	23 x LS	57.32[ab]	1.25	2.06	3.01	4.08[b]
	23 x A	48.46[d]	1.37	2.44	3.13	5.16[a]
	23 x LB	57.34[ab]	1.37	2.43	3.37	4.16[b]
	4 x LS	60.27[a]	0.24	0.63	1.08	1.56[c]
	4 x A	51.61[cd]	0.29	0.75	1.28	1.84[c]
	4 x LB	55.57[bc]	0.28	0.79	1.14	1.61[c]
	SEM	1.101	0.062	0.089	0.094	0.128
	P-value	0.045	0.794	0.291	0.120	0.004

[a-d] Differences between groups indicated with different letters in the same column are statistically significant (P<0.05). LS: Lohmann Sandy genotype, A: ATAK-S genotype, LB: Lohmann Brown genotype, SEM: Standard error of mean.

Haugh Unit

The effect of storage temperature, genotype, and storage x genotype interaction on egg Haugh units is shown in Table 2. The interaction effects of storage temperature x genotype on egg albumen height were found to be statistically insignificant at all periods of storage. The effects of storage temperature on the egg Haugh unit were found to be statistically insignificant only in the before storage Haugh unit and were found to be statistically significant in all periods of storage (P<0.05). In general, the Haugh unit was found to be higher in eggs stored at 4 °C compared to eggs stored at 25 °C, depending on the temperature in all periods of storage (P<0.05). On the 28th day of storage, the Haugh unit was 77.96 in eggs stored at 4 °C, while the Haugh unit in eggs stored at 23 °C was 74.29 (P<0.05). The effects of genotype on the egg Haugh unit were found to be statistically significant in the before storage Haugh unit and at all periods of storage (P<0.05). In the before storage Haugh unit, on the seventh (7) and twenty-eighth (28) days of storage, Lohmann Brown genotype eggs were higher than Haugh unit (99.18), ATAK-S genotype (93.92), and Lohmann Sandy genotype eggs (94.23) (P<0.05). However, the difference between Lohmann Sandy genotype (94.23) and Haugh units (93.92) of ATAK-S genotype eggs was found to be statistically insignificant. On the fourteenth (14) and twenty-one (21) days of storage, the Haugh unit of Lohmann Brown genotype eggs (day 14: 93.20, day 21: 87.09), ATAK-S (day 14: 82.75, day 21: 76.22), and Lohmann Sandy genotype eggs derived from the Haugh unit (day 14: 87.52, day 21: 80.53) was found to be higher (P< 0.05). Similarly, the Haugh unit of Lohmann Sandy genotype eggs (14 day: 87.52, 21.day: 80.53) was found to be higher than the Haugh unit (14 day: 82.75, 21 day: 76.22) of ATAK-S genotype eggs (P<0.05). On the 28th day of storage, the Haugh unit (82.23) of the Lohmann Brown genotype eggs was found to be higher than the Haugh unit (70.48) of the ATAK-S genotype and the Haugh unit (75.68) of the Lohmann Sandy genotype eggs (P<0.05). No significant differences were found between the Haugh unit of the ATAK-S genotype (70.48) and the Haugh unit of the Lohmann Sandy genotype (75.68).

Table 2. Effect of storage temperature, genotype and storage x genotype interaction on Haugh unit

Treatment		Before Storage Haugh Unit	Haugh Unit			
			7 d	14 d	21 d	28 d
Storage temperature (°C)	23	94.72	88.98[b]	84.43[b]	79.81[b]	74.29[b]
	4	96.83	93.61[a]	91.21[a]	82.75[a]	77.96[a]
	SEM	1.210	0.891	1.110	0.995	1.231
	P-value	0.221	0.001	0.000	0.041	0.039
Genotype	LS	94.23[b]	90.72[b]	87.52[b]	80.53[b]	75.68[b]
	A	93.92[b]	87.76[b]	82.75[c]	76.22[c]	70.48[b]
	LB	99.18[a]	95.42[a]	93.20[a]	87.09[a]	82.23[a]
	SEM	1.481	1.090	1.358	1.218	1.507
	P-value	0.022	0.000	0.000	0.000	0.000
Storage temperature (°C) x Genotype	23 x LS	93.20	87.99	84.07	79.21	73.82
	23 x A	91.75	85.70	78.60	74.77	70.66
	23 x LB	99.20	93.26	90.63	85.47	78.40
	4 x LS	95.26	93.44	90.97	81.85	77.53
	4 x A	96.08	89.82	86.89	77.67	70.29
	4 x LB	99.15	97.58	95.77	88.72	86.06
	SEM	2.094	1.542	1.920	1.721	2.130
	P-value	0.590	0.895	0.720	0.984	0.188

[a-c] Differences between groups indicated with different letters in the same column are statistically significant (P<0.05). LS: Lohmann Sandy genotype, A: ATAK-S genotype, LB: Lohmann Brown genotype, SEM: Standard error of mean

Yolk Index

The effect of storage temperature, genotype and storage x genotype interaction on egg yolk index is shown in Table 3. The interaction effects of storage temperature x genotype on egg yolk index were found to be statistically significant only on the 14 d and 28 d of storage (P<0.05). On the 14 d of storage, the highest yolk index (0.57) was observed in the eggs of the Lohmann Brown genotype stored at 4 °C, and the lowest yolk index value was observed in the eggs of the ATAK-S (0.42) genotype stored at 23 °C. On the 28th day of storage, the highest yolk index (0.47) was observed in the eggs of the Lohmann Sandy genotype stored at 4 °C, and the lowest yolk index value was observed in the eggs of the Lohmann Brown genotype (0.33) stored at 23 °C. However, the difference between the yolk index (0.40) of Lohmann Sandy genotype eggs stored at 23°C and the yolk index (0.43) of ATAK-S genotype eggs stored at 4°C was found to be statistically insignificant. The effects of temperature on the egg yolk index were found to be statistically insignificant in the before storage yolk index and were found to be statistically significant in all periods of storage (P<0.05). In general, the yolk index was found to be higher in eggs stored at 4 °C in all periods compared to eggs stored at 23 °C (P<0.05). While the yolk index was 0.46 in eggs stored at 4 °C on the 28th day of storage, the yolk index was 0.35 in eggs stored at 23 °C (P<0.05). The effects of genotype on egg yolk index were found to be statistically insignificant in the before storage yolk index and were found to be statistically significant in all periods of storage (P<0.05). On the 7 d of storage, the yolk index of the ATAK-S genotype was lower than the yolk index of the Lohmann Sandy genotype (P<0.05), it was similar to the yolk index of the Lohmann Brown genotype, and the difference in yolk index between the eggs of the Lohmann Brown and Lohmann Sandy genotypes was statistically significant. was found to be insignificant. On the 14 d of storage, the yolk index of the ATAK-S genotype was lower than the yolk index of the Lohmann Sandy genotype and Lohmann Brown genotype (P<0.05), and the difference between the yolk index value of the eggs of the Lohmann Brown and Lohmann Sandy genotypes was statistically insignificant. On the twenty-first day of storage, the yolk index of the ATAK-S genotype was lower than the yolk index of the Lohmann Sandy genotype (P<0.05), it was similar to the yolk index of the Lohmann Brown genotype, and the yolk index of the Lohmann Brown genotype eggs was found to be lower than the yolk

index of the Lohmann Sandy genotype eggs. (P<0.05). On the 28th day of storage, the yolk index (0.44) of the eggs of the Lohmann Sandy genotype was higher than the egg yolk index (0.38) of the ATAK-S genotype and the yolk index (0.40) of the Lohmann Brown genotype (P<0.05). There was no statistical difference between the yolk index (0.38) and the egg yolk index (0.40) of the Lohmann Brown genotype.

Table 3. Effect of storage temperature, genotype and storage x genotype interaction on yolk index

Treatment		Before storage yolk index	Yolk index			
			7 d	14 d	21 d	28 d
Storage temperature(°C)	23	0.55	0.50^{b}	0.44^{b}	0.40^{b}	0.35^{b}
	4	0.57	0.55^{a}	0.54^{a}	0.50^{a}	0.46^{a}
	SEM	0.0068	0.0053	0.0045	0.0050	0.0063
	P-value	0.094	0.000	0.000	0.000	0.000
Genotype	LS	0.57	0.54^{a}	0.51^{a}	0.49^{a}	0.44^{a}
	A	0.55	0.51^{b}	0.46^{b}	0.43^{b}	0.38^{b}
	LB	0.56	0.53^{ab}	0.50^{a}	0.44^{b}	0.40^{b}
	SEM	0.0083	0.0065	0.0055	0.0061	0.0077
	P-value	0.420	0.002	0.000	0.000	0.000
Storage temperature(°C) x Genotype	23 x LS	0.56	0.52	0.45^{c}	0.44	0.40^{b}
	23 x A	0.54	0.48	0.42^{d}	0.38	0.34^{c}
	23 x LB	0.57	0.50	0.44^{cd}	0.39	0.33^{c}
	4 x LS	0.58	0.57	0.56^{a}	0.53	0.47^{a}
	4 x A	0.57	0.53	0.50^{b}	0.48	0.43^{ab}
	4 x LB	0.56	0.55	0.57^{a}	0.49	0.46^{a}
	SEM	0.0116	0.0092	0.0465	0.0087	0.0109
	P-value	0.127	0.846	0.041	0.913	0.017

[77]

[a-d] Differences between groups indicated with different letters in the same column are statistically significant (P<0.05). LS: Lohmann Sandy genotype, A: ATAK-S genotype, LB: Lohmann Brown genotype, SEM: Standard error of mean.

Albumen pH

The effect of storage temperature, genotype and storage x genotype interaction on egg albumen pH is shown in Table 4. The interaction effects of storage temperature x genotype on egg yolk index were found to be statistically insignificant in all periods of the experiment. The effects of temperature on egg albumen pH were found to be statistically insignificant at the day 0 of storage and were found to be statistically significant in all periods of storage (P<0.05). In general, albumen pH was found to be lower in eggs stored at 4 °C in all periods compared to eggs stored at 23 °C (P<0.05). On the 28th day of storage, the albumen pH value was 8.96 in eggs stored at 4 °C, while the albumen pH value in eggs stored at 23 °C was 9.12 (P<0.05). The effects of genotype on egg albumen pH were found to be statistically significant at 14, 21 and 28 days of storage (P<0.05). On the 14 d of storage, the albumen pH value of Lohmann Sandy genotype eggs (8.89) was lower than the albumen pH value of ATAK-S genotype eggs (8.97). On the 21d of storage, the albumen pH value of Lohmann Sandy genotype eggs (8.93) was lower than the albumen pH value of ATAK-S genotype eggs (9.04) and the albumen pH value of Lohmann Brown genotype eggs (9.02) (P<0.05). On the 28th day of storage, the albumen pH value of ATAK-S genotype eggs (9.06) was higher than the albumen pH value of Lohmann Sandy genotype eggs (9.03) and the albumen pH value of Lohmann Brown genotype eggs (9.03) (P<0.05). The difference between the albumen pH value of eggs obtained from the Lohmann Brown genotype (9.03) and the albumen pH value of eggs obtained from the Lohmann Sandy genotype (9.03) was found to be statistically insignificant.

Table 4. Effect of storage temperature, genotype and storage x genotype interaction on albumen pH

Treatment		Before storage albumen pH	Albumen pH			
			7 d	14 d	21 d	28 d
Storage temperature (°C)	23	8.57	8.98[a]	9.02[a]	9.07[a]	9.12[a]
	4	8.60	8.80[b]	8.84[b]	8.92[b]	8.96[b]
	SEM	0.044	0.020	0.014	0.010	0.008
	P-value	0.663	0.000	0.000	0.000	0.000
Genotype	LS	8.52	8.85	8.89[b]	8.93[b]	9.03[b]
	A	8.68	8.91	8.97[a]	9.04[a]	9.06[a]
	LB	8.56	8.91	8.94[ab]	9.02[a]	9.03[b]
	SEM	0.054	0.024	0.018	0.012	0.010
	P-value	0.097	0.178	0.010	0.000	0.024
Storage temperature (°C) x Genotype	23 x LS	8.54	8.91	8.95	8.97	9.12
	23 x A	8.69	9.01	9.08	9.12	9.14
	23 x LB	8.49	9.01	9.04	9.10	9.11
	4 x LS	8.50	8.80	8.83	8.86	8.93
	4 x A	8.67	8.80	8.85	8.96	8.98
	4 x LB	8.63	8.81	8.85	8.94	8.95
	SEM	0.076	0.035	0.025	0.017	0.014
	P-value	0.435	0.346	0.077	0.504	0.433

[a-b] Differences between groups indicated with different letters in the same column are statistically significant (P<0.05). LS: Lohmann Sandy genotype, A: ATAK-S genotype, LB: Lohmann Brown genotype, SEM: Standard error of mean

Discussion

Egg Weight Loss

As a result of the research, the effect of temperature on egg weight loss during storage was found to be statistically significant. This result is consistent with the studies of Gavril and Usturoi (2011), Tayeb (2012), Akter et al., (2014), Jones et al., (2018), and Kale and Aygün (2022). Jones et al., (2018) found that on the 28th day of storage, egg weight loss was 0.58% in eggs stored at 4 °C, and 4.67% in eggs stored at 23 °C. Tayeb (2012) determined the weight loss of eggs as 7.66% in eggs stored at room temperature (25-30°C) on the 27th day of storage, and as 2.93% in eggs stored in a refrigerator (5 °C). In a study by Gavril and Usturoi (2011) they found that egg weight loss was 1.99% at 4°C and 3.12% at 25°C in eggs stored at 4 °C and 25 °C. Kale and Aygün (2022) determined that the average egg weight loss at the end of the 28th day of storage was 1.53% in eggs stored at 4°C, and 5.68% in eggs stored at 23°C at the end of the 28th day of storage. Akter et al., (2014) determined that after 28 days of storage, egg weight loss in eggs stored at 4 °C was lower than in eggs stored at 28-31 °C.

As a result of the research, the effect of genotype on egg weight loss was found to be significant on the 14 and 28 days of storage. The egg weight loss of the ATAK-S genotype was found to be higher than that of the Lohmann Brown and Lohmann Sandy genotypes on the 28th day of storage. This result is compatible with the studies of Silversides et al., (2001), Tunçer (2006), Şekeroğlu et al., (2008), Bozkurt and Tekerli (2009), and Alsobayel and Albadry (2011). Alsobayel and Albadry (2011) found in their study that the white shelled egg weight was higher than the brown shelled egg weight after 20 days of storage of eggs. Silversides et al., (2001) determined that the egg weight of the ISA-Brown genotype was higher than the egg weight of the ISA-White genotype after 10 days of storage. In the study conducted by Tunçer (2006), it was determined that the egg weight of the Isa-Brown genotype was higher than the egg weight of the Babcock300 genotype after 14 days of storage.

The cuticle layer on the eggshell, synthesized by the secretory cells 1.5-2 hours before ovulation, acts as a buffer for gas and water permeability in the egg (Wyburn et al., 1973; Nys et al., 1999; Samiullah et al., 2014; Ketta and Tůmová 2016; Wilson et al., 2017). The permeability of the crust increases with the drying of the cuticle (Rodríguez-Navarro et al., 2013). During storage, egg weight loss occurs when the water vapor in the egg is removed from the egg through the pores

densely located in the egg shell (Akyurek and Okur 2009). It is thought that the rate of removal of the water spring increases at higher temperatures.

Haugh Unit

In our study, the effect of temperature on the Haugh Unit was found to be significant as a result of storing eggs obtained from different layer genotypes (Lohmann Sandy, Lohmann Brown and ATAK-S) at different temperatures (4 ℃ and 23 ℃). This result is similar to Samli et al., (2005), Bozkurt and Tekerli (2009), Baylan et al., (2011), Jin et al., (2011), Gavril and Usturoi (2011), Adamski et al., (2017), Kale and Aygün (2022). It is compatible with the studies conducted by Samli et al., (2005) conducted a study to determine the effects of storage temperature on the quality parameters of eggs obtained from Bovans White egg hens, and as a result of 10 days of storage, the Haugh unit of eggs stored at 5 °C was (76.27) compared to that of eggs stored at 21 °C. They found that the Haugh unit (53.74) of eggs stored at 29°C was higher than the Haugh unit (53.74). In a study carried out by Bozkurt and Tekerli (2009) to examine the egg quality characteristics of eggs obtained from different layer genotypes depending on storage conditions, the Haugh unit of eggs stored at 4 °C (58.11) after 5 weeks of storage was lower than the Haugh unit of eggs stored at 24 °C (58.11). 40.90) were found to be higher. Jin et al., (2011) conducted a study to determine the effect of storage temperature on egg quality. As a result of 10 days of storage, the Haugh unit of eggs stored at 5 °C was 87.63, the Haugh unit of eggs stored at 21 °C was 72.63, and the Haugh unit of eggs stored at 29 °C was 87.63. It was determined to be higher than the Haugh unit (61.85) of stored eggs. Gavril and Usturoi (2011), in their study to determine the effect of the level of environmental factors provided during egg storage on egg quality, at the end of the 28th day of storage, the Haugh unit of eggs stored at 4 °C (73.48), was lower than the Haugh unit of eggs stored at 25 °C (48.45), were found to be higher. In their study to examine the change in egg quality characteristics depending on storage conditions, Adamski et al., (2017) found that the Haugh unit of eggs stored at 4 °C (71.60) after 28 days of storage was higher than the Haugh unit (32.66) of eggs stored at 23 °C were found to be high. Kale and Aygün (2022) examined the effect of storing eggs obtained from different rearing systems at different temperatures on egg quality. According to the results obtained from the study, they determined that the Haugh unit of eggs stored at 4 °C (69.81) was higher than the Haugh unit of eggs stored at 23 °C (62.98) after 28 days of storage.

As a result of our study, the effect of genotype on the Haugh Unit was found to be significant. On the 28th day of storage, it was determined that the Haugh unit value (82.23) of Lohmann Brown genotype eggs was higher than the Haugh unit value of ATAK-S genotype eggs (70.48) and the Haugh unit value of Lohmann Sandy genotype eggs (75.68) ($P<0.05$), while the Lohmann Sandy genotype egg Haugh unit value (70.48) was higher than the Haugh unit value (75.68) ($P<0.05$). The Haugh unit of Lohmann Sandy genotype eggs is similar to the Haugh unit of ATAK-S genotype eggs. While this result is parallel to the studies of Tunçer (2006), Bozkurt and Tekerli (2009), and Şekeroğlu et al., (2008), it is incompatible with the study of Alsobayel and Albadry (2011). Tunçer (2006) examined the effect of storage on egg quality criteria in two different commercial laying hen genotypes (Babcock300 and Isa-Brown), and in the study, the effect of genotype on the Haugh unit was found to be significant. As a result of their research, Bozkurt and Tekerli (2009) found the effect of genotype on the Haugh Unit in storage in two different laying hen genotypes (Lohmann White and ISA Brown) to be significant. At 5 weeks of storage, the Haugh unit (51.96) of eggs belonging to the Lohmann White genotype was determined to be higher than the Haugh unit (47.05) of eggs belonging to the Isa Brown genotype. Şekeroğlu et al., (2008) found in their study that the effect of genotype on the Haugh unit was significant as a result of the storage of eggs obtained from ATAK and ATABEY genotypes. They found that the Haugh unit of ATABEY genotype eggs (74.40) was higher than the Haugh unit of ATAK genotype eggs (77.10) on the 20th day of storage. In a study conducted by Alsobayel and Albadry (2011), the effect of genotype on storage of eggs obtained from brown and white laying hens was found to be insignificant. On the 20th day of storage, the Haugh unit (79.48) of the eggs obtained from the brown layer genotype and the Haugh unit (79.39) of the eggs obtained from the white layer genotype were determined to be similar.

The decrease in Haugh unit occurred due to decreases in albumen height and increased egg weight loss. At high storage temperatures, denaturations in the structure of ovomucin, the egg albumen protein, occur rapidly, and the albumen height decreases due to the decrease in egg albumen density (Tunçer 2006; Quan and Benjakul 2018; Quan and Benjakul 2019). Similarly, high storage temperatures increase egg weight losses by affecting the evaporation rate of water vapor in the egg.

Yolk index

In our study, the effect of temperature on the yolk index was found to be significant in all periods of storage. This finding supports parallelism between research by Samli et al., (2005), Akyurek and Okur (2009), Bozkurt and Tekerli (2009), Gavril and Usturoi (2011), Tayeb (2012), Akpinar et al., (2015), and Jones et al., (2018). Gavril and Usturoi (2011) found the effect of temperature on the yolk index to be significant. At the end of the 28th day of storage, the yolk index (0.36) of eggs stored at 4 °C was found to be higher than the yolk index (0.28) of eggs stored at 25 °C. Jones et al., (2018) found that, after 6 weeks of storage, the yolk index of eggs stored at 4 °C (0.54) was higher than the yolk index of eggs stored at 25 °C (0.35). Bozkurt and Tekerli (2009) found that the yolk index (0.55) of eggs stored at 4 °C was higher than the yolk index (0.45) of eggs stored at 24 °C in the 5th week of storage. Akyurek and Okur (2009) found that the

yolk index of eggs stored at 4°C was higher than the yolk index of eggs stored at 20°C after 14 days of storage. Samli et al., (2005) examined the effect of different storage temperatures (5 °C, 21 °C and 29 °C) and storage time on egg quality in laying hens and found the effect of temperature on the yolk index to be significant. They found that after 10 days of storage, the yolk index of eggs stored at 5 °C was higher than the yolk index of eggs stored at 21 °C and the yolk index of eggs stored at 29 °C. Tayeb (2012) found the effect of temperature on yolk index to be significant. On the 27th day of storage, the yolk index of eggs stored at 5 °C was determined to be higher than the yolk index of eggs stored at 25-30 °C.

In our study, the effect of genotype on yolk index was found to be significant in all periods of storage. Nevertheless, this result is parallel to the studies of Bozkurt and Tekerli (2009), and (Keener et al., 2006), it is opposition to those of Şekeroğlu et al., (2008). As a result of their research, Bozkurt and Tekerli (2009) found that the effect of genotype on the yolk index in storage in two different laying hen genotypes (Lohmann White and ISA Brown) was significant. They found that the yolk index of the Lohmann White genotype (0.48) was lower than the yolk index of the ISA Brown genotype (0.50) in eggs stored for 5 weeks. In a study conducted by Keener et al., (2006), the effects of genotype on yolk index were found to be significant as a result of storage of two different chicken genotypes (Hyline White 36 and Bovans White). The yolk index (0.45) of the Bovans White genotype was determined to be higher than the yolk index of the Hyline White-36 genotype in 7-week-old stored eggs. Şekeroğlu et al., (2008) found that the effect of genotype on the yolk index as a result of storage of eggs obtained from ATAK and ATABEY chickens was insignificant. After 20 days of storage, the yolk index (0.40) of ATABEY genotype eggs and the yolk index (0.40) of ATAK genotype eggs were determined to be similar.

As a result of the decrease in the amount of albumen and deterioration in its structure during storage, the yolk loses its spherical appearance and acquires a round and loose appearance, causing the yolk diameter to increase (Silversides and Budgell 2004). As the vitelline membrane in the egg albumen loses its elasticity, it ruptures and the egg albumen and yolk mix (Avan and Alişarlı 2002). Deterioration in the structure of the vitelline membrane causes the yolk height to decrease and the yolk diameter to increase. Accordingly, decreases occur in the yolk index (Kale and Aygün 2022).

Albumen pH

In our study, the effect of temperature on albumen pH was found to be important in all periods of storage. This result is in parallel with those of Jin et al., (2011), Chung and Lee (2014), Lee et al., (2016), Adamski et al., (2017) and Feddern et al., (2017). Jin et al., (2011) found that after 10 days of storage, the albumen pH value of eggs stored at 5 °C (8.76) was lower than the albumen pH value of eggs stored at 21 °C (9.50) and the albumen pH value of eggs stored at 29 °C (9.71). Chung and Lee (2014) found that the albumen pH value (8.72) of eggs stored at 4 °C on the 28th day of storage was lower than the albumen pH value (9.03) of eggs stored at 23 °C. In a study conducted by Lee et al., (2016), on the 30th day of storage, the albumen pH value of eggs stored at 2°C (8.03) was compared with the albumen height value of eggs stored at 12°C (8.68) and the albumen pH value of eggs stored at 25°C (8.68). As a result of 28 days of storage, Adamski et al., (2017) found that the albumen pH value of eggs stored at 4 °C (8.26) was lower than that of eggs stored at 23 °C (8.54). Furthermore, Feddern et al., (2017) found that the albumen pH value of eggs stored at 5°C (8.63) on the 28th day of storage was higher than the albumen pH value of eggs stored at 20-30°C (9.30).

In our study, the effect of genotype on albumen pH was found to be significant on the 14th, 21st and 28th days of storage. On the 28th day of storage, the albumen pH value of ATAK-S genotype eggs (9.06) was higher than the albumen pH value of Lohmann Sandy genotype eggs (9.03) and the albumen pH value of Lohmann Brown genotype eggs (9.03). The albumen pH value of Lohmann Brown genotype eggs and the albumen pH value of Lohmann Sandy genotype eggs were found to be similar. While this result is parallel to those of Silversides and Budgell (2004) and Şekeroğlu et al., (2008), it is incompatible with the study conducted by Feddern et al., (2017). In their study by Silversides and Budgell (2004), the effect of genotype on albumen pH was found to be significant as a result of storage of eggs obtained from Brown Leghorn, ISA Brown, Babcock genotypes.

After 10 days of storage, the albumen pH value of the Brown Leghorn genotype (8.84) was determined to be higher than the Bobcook genotype albumen pH value (8.70) and the albumen pH value of the ISA Brown genotype (8.67), and the difference between ISA Brown and Babcock genotype eggs was found to be statistically significant. Şekeroğlu et al., (2008) found that the effect of genotype on albumin pH was significant as a result of storage of eggs obtained from ATAK and ATABEY genotypes. After 20 days of storage, the albumen pH value of ATAK genotype eggs (7.62) was higher than that of ATABEY genotype eggs (7.54). As a result of storage of eggs obtained from white and brown layer genotypes, the effect of genotype on albumen pH was found to be insignificant by Feddern et al., (2017). After 28 days of storage, the albumen pH value of eggs obtained from brown genotypes (8.94) was determined to be similar to the albumen pH value of eggs obtained from white genotypes (8.98).

During storage, the ovomucin-lysozyme complex breaks down and helps increase the pH of the eggs (Akter et al., 2014). High storage temperatures cause rapid removal of water and CO2 from the egg albumen through the pores in the eggshell, resulting in greater increases in albumen pH (Avan and Alişarlı 2002; Yılmaz and Bozkurt 2008).

Conclusions

The genotype x storage temperature interaction effect did not generally significantly affect egg quality characteristics during storage. It has been observed that the quality characteristics of eggs stored under refrigerator conditions during storage are better than those stored under room conditions. During storage, the quality characteristics of eggs obtained from the ATAK-S genotype were observed to be in worse condition than the eggs of the Lohmann Brown and Lohmann Sandy genotypes. Refrigerator should be preferred for storing table eggs. It should be taken into consideration that genotype has a significant impact on the storage of table eggs.

Author Contributions:

Conceptualization, C.B. and A.A.; methodology, C.B. and A.A.; validation, C.B. and A.A; formal analysis, C.B., and A.A.; investigation, C.B. and A.A.; data curation, C.B., A.A. and F.İ.; writing—original draft preparation, C.B. and A.A.; writing—review and editing, C.B., and A.A.; supervision, C.B., A.A.; project administration, A.A.; funding acquisition, A.A. All authors have read and agreed to the published version of the manuscript.

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POULTRY STUDIES

RESEARCH PAPER

Effects of Environmental Enrichment on Ostrich Behaviours

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Abstract

This study was aimed to investigate the behavioural differences of ostrich (*Struthio camelus*) breeders reared under environmental enrichment with natural vegetation in farm conditions. The effect of enriched environment with vegetation on eating, drinking, foraging, pecking, defecation, walking, running, alert, standing, sitting, sleeping, dust bathing, preening, head shake, thermoregulation, kantel, mating, laying, threat and fight behaviours of ostriches were found similar (P > 0.05). The ratio of boom, courtship and displace behaviours were found higher in enriched than in gravel floor (P=0.050; P=0.028 and P=0.001). The foraging, dust bathing, boom, laying and displace behaviours of ostriches were affected by gender (P=0.029, P=0.040, P=0.050, P=0.025 and P=0.001). The eating, foraging, standing, sitting, dust bathing and laying behaviours of ostriches were affected by time of day (P=0.010, P=0.023, P=0.049, P=0.026, P=0.018 and P=0.009). There was a significant interaction of enriched environment and gender effect on eating, standing, boom and displace behaviours of ostriches (P=0.047, P=0.031, P=0.050 and P=0.001). The pecking and standing behaviours of ostriches were affected by enriched environment and time of day interaction (P=0.027 and P=0.023). As a conclusion, enriched environment with natural vegetation in paddock only affected ostriches' courtship behaviours, also affected male and female eating and standing behaviours differently.

Introduction

The ostrich is the largest and heaviest living bird (150 kg and 2.50 m in height), and it is a member of the ratite family of flightless runners. The ostrich is an African native bird that lives in semiarid plains and woodlands (Deeming, 1999). In the 1880s, commercial ostrich production began in the Africa Oudtshoorn region for feather manufacture. In the years that followed, manufacturing of its skin, eggs, and meat spread throughout the world (Şahan et al., 2000).

The definition of behaviour is a specific response to environmental variables. Ostriches in their native habitat exhibit unique behavioural patterns that allow them to adapt to their surroundings. However, in captivity, behavioural tendencies may alter due to environmental variables (Hambali et al., 2015). Ostriches, raised in farm circumstances that differ from their natural environments, are subjected to extreme stress, which leads to yield losses (Amado et al., 2011; Muvhali, 2018).

Welfare problems are commonly found in intensive farming systems. It has been recommended to create environment that are interesting and enriching in order to lessen the severity of welfare concerns. Environmental enrichment is the process of enhancing an animal's surroundings in captivity so that it has more chances for behaviour and has better biological function (Riber et al., 2018). According to Newberry (1995), environmental enrichment should increase the animal's ability to cope with behavioural and physiological challenges. And, according to Van de Weerd & Day (2009) the successful enrichment must fulfill as follows; it should promote species-specific

behaviour, uphold or enhance health levels, enhance the production system's profitability, and be useful to use. Materials such as peck objects, perches, barriers, bales, and materials that stimulate foraging and dust bathing behaviours, outdoor access areas (free-range systems etc.), outdoor natural or artificial cover, sheds, and shades (bushes, trees etc.) are used for environmental enrichment in layer and broiler rearing (Riber et al., 2018).

On farms all around the world, ostrich farming is done and propagated using a variety of different, sometimes contradictory ways. The majority of ostriches in South Africa are kept on steppe soil or in semi-desert areas with sparse vegetation, and they are given either farm products or commercially produced feed (Kistner, 2019). Most of the world ostriches are kept in paddocks in intensive farming systems which is different from bird's natural environment (Cooper, 2000). Although there were some studies about environmental enrichment with vegetation on behaviours of broilers and layers (Jones et al., 2007; Almeida et al., 2012; Dal Bosco et al., 2014), there is limited published scientific research about environmental enrichment on behaviours of ostriches and rheas (Christensen & Nielsen, 2004; Lima et al. 2019). The aim of the study was to investigate the effects of environmental enrichment with vegetation on behaviour of male and female ostrich (Struthio camelus) breeders reared under intensive farming system to provide the useful information for improved management.

Materials and Methods

The study was conducted on 4 female and 2 male ostrich (*Struthio camelus*) breeder at the Bursa Uludağ University Research and Application Farm unit. Ostriches reared in trio; 1 male and 2 female in one paddock. One paddock contained 1000 m2 floor area, 5 m2 shelter area, 3 m high wire fence, feeders, drinkers and grass feeders. The paddock floor was covered with soil and gravel. Natural vegetation (such as; *Silybum marianum, Rumex ssp., Malvae sylvestris, Xanthium strumarium, Bromus tectorum, Cynodon dactylon*) grows in paddocks over time and the vegetation routinely cut. For this study, one paddock floor vegetation left uncut and left to its natural state for one year period. Two similar sized paddocks used in the study one paddock was used as an envrionmetal enrichment with natural vegetation group, the other paddock was used as gravel floor group (Control). All ostriches were fed with 2 kg/bird per day of a pellet ostrich breeder feed (18% CP, 2450 kcal ME) and 500 g/bird per day alfalfa. Water was supplied ad libitum. Natural sun lighting was used.

Behaviour recordings were taken with binoculars and naked eye by one trained person. The observer waits for before recording the behaviours for one hour. A scan sampling method was used to monitor the behaviours of birds as described by Mitlöhner et al. (2001). For each bird, behavioural observations were recorded at 10 min intervals for 1 h in morning, noon and evening (at 09:00; 13:00 and 17:00; respectively). All the birds were monitored for six days. The individual behaviours were recorded as eating, drinking, foraging, defecation, walking, running, alert, pecking, standing, sitting, sleeping, dust bathing, preening, head shake, thermoregulation, boom, courtship, kantel, mating, laying, threat, displace, fight, escape described in Stewart (1994) and Amado et al. (2011).

The data was analyzed using PROC MIXED procedure of Statistical Analysis System (SAS, 2019). The model included the fixed effects of gender (male and female), environment (gravel and vegetation floor), and time of day (morning, noon and evening), replicate and all interactions. Individual bird number within each replicate was entered as a random factor. Data were presented as mean ± standard error (SE) in all the tables. Differences were considered significant at $P \leq 0.05$ and the statistical difference at $P < 0.10$ was described as a tendency. The statistical model was as follows:

$Y_{ij} = \mu + a_i + b_j + c_k + (ab)_{ij} + (ac)_{ik} + (abc)_{ijk} + \varepsilon_{ijk}$, where $Y_{ij} = \mu^{th}$ observation value, μ = expected mean of the population, a_i = i. enrichment effect (i=gravel and vegetation), b_j = j. gender effect (j= male and female), c_k = k. time of day effect (k= morning, noon and evening), $(ab)_{ij}$ = ij. enrichment and gender interaction effect, $(ac)_{ik}$ = ik. enrichment and time of day interaction effect, $(abc)_{ijk}$ = ijk. enrichment and gender and time of day interaction effect, ε_{ijk} = residual error.

Results

The effect of enriched environment with natural vegetation on ingestive behaviours of ostriches were given in Table 1. The effect of enriched environment on eating, drinking, foraging, pecking and defecation behaviour was not significant ($P > 0.05$). The gender of ostrich effected foraging behaviour, and higher foraging behaviour was observed in females ($P=0.029$). The difference in eating, drinking, pecking and defecation behaviour of male and female ostriches were similar during the study ($P > 0.05$). The eating and foraging behaviours of ostriches were affected by time of day ($P=0.01$ and $P=0.023$, respectively). The lowest eating behaviour was observed at noon, and the highest foraging behaviour was found in the morning. The drinking behaviour tends to be higher in the morning and at noon ($P=0.055$). The pecking and defecation behaviour was not changed during the day ($P > 0.05$). The effect of enriched environment with vegetation and gender interaction on eating behaviour of ostrich was found significant ($P = 0.047$), lowest eating behaviour was found in males in enrichment group. The effect of enriched environment and gender interaction on drinking, foraging, pecking and defecation behaviours of ostriches were not significant ($P > 0.05$). The effect of enriched environment and time of day interaction on ingestive behaviours of ostriches were found not significant ($P > 0.05$); except pecking behaviour ($P=0.027$). The higher pecking behaviour was observed during the evening in gravel group and during the

Table 1. Effects of enriched environment on ingestive behaviours of ostriches (number of bouts/hour)

Enrichment		Eating	Drinking	Foraging	Pecking	Defecation
Floor	Gravel	0.13 ± 0.02	0.021 ± 0.01	0.10 ± 0.02	0.07 ± 0.02	0.03 ± 0.01
	Vegetation	0.11 ± 0.02	0.014 ± 0.01	0.09 ± 0.02	0.05 ± 0.02	0.01 ± 0.01
P		*0.593*	*0.453*	*0.728*	*0.344*	*0.220*
Gender	Male	0.12 ± 0.02	0.011 ± 0.01	0.07 ± 0.02^{b}	0.06 ± 0.01	0.01 ± 0.01
	Female	0.12 ± 0.02	0.024 ± 0.01	0.13 ± 0.02^{a}	0.07 ± 0.01	0.02 ± 0.01
P		*0.995*	*0.167*	*0.029*	*0.661*	*0.424*
Time of Day	MO	0.14 ± 0.02^{a}	0.035 ± 0.01^{a}	0.15 ± 0.02^{a}	0.08 ± 0.02	0.03 ± 0.01
	NO	0.06 ± 0.02^{b}	0.012 ± 0.01^{ab}	0.08 ± 0.02^{b}	0.05 ± 0.02	0.01 ± 0.01
	EV	0.16 ± 0.02^{a}	0.007 ± 0.01^{b}	0.07 ± 0.02^{b}	0.06 ± 0.02	0.02 ± 0.01
P		*0.010*	*0.055*	*0.023*	*0.628*	*0.471*
Floor X Gender						
G X Male		0.16 ± 0.03^{a}	0.017 ± 0.01	0.07 ± 0.02	0.07 ± 0.02	0.03 ± 0.01
G X Female		0.10 ± 0.03^{ab}	0.026 ± 0.01	0.13 ± 0.02	0.07 ± 0.02	0.02 ± 0.01
V X Male		0.08 ± 0.03^{b}	0.006 ± 0.01	0.07 ± 0.02	0.04 ± 0.02	-
V X Female		0.14 ± 0.03^{a}	0.023 ± 0.01	0.12 ± 0.02	0.06 ± 0.02	0.02 ± 0.01
P		*0.047*	*0.681*	*0.728*	*0.649*	*0.220*
Floor X Time of day						
G X MO		0.14 ± 0.03	0.05 ± 0.01	0.13 ± 0.03	0.05 ± 0.02^{ab}	0.03 ± 0.01
G X NO		0.07 ± 0.03	0.02 ± 0.01	0.11 ± 0.03	0.07 ± 0.02^{ab}	0.01 ± 0.01
G X EV		0.16 ± 0.03	-	0.07 ± 0.03	0.10 ± 0.02^{a}	0.04 ± 0.01
V X MO		0.14 ± 0.03	0.02 ± 0.01	0.14 ± 0.03	0.10 ± 0.02^{a}	0.03 ± 0.01
V X NO		0.04 ± 0.03	0.01 ± 0.01	0.06 ± 0.03	0.04 ± 0.02^{ab}	0.01 ± 0.01
V X EV		0.16 ± 0.03	0.02 ± 0.01	0.07 ± 0.03	0.02 ± 0.02^{b}	0.01 ± 0.01
P		*0.893*	*0.255*	*0.357*	*0.027*	*0.529*
Floor X Gender X Time of Day						
P		*0.696*	*0.903*	*0.999*	*0.606*	*0.821*

^{a,b}: The different superscripts on numbers represent a significant difference between them (*P*<0.05).
Data are presented as LSM ± SEM.
G: Gravel, V: Vegetation, MO: Morning, NO: Noon, EV: Evening, - : No Behaviour.

morning in enrichment group. The three-way interaction effect of enriched environment, gender and time of day on ingestive behaviours of ostrich was not significant (P > 0.05).

The effects of enriched environment with natural vegetation on locomotor and resting behaviours of ostriches were given in Table 2. The effect of enriched environment on walking, running, alert, standing, sitting, sleeping behaviours of ostrich was not significant (P > 0.05). The gender of ostrich tends to be affected alert and standing behaviour, and higher alert and standing behaviour was observed in males (P=0.059 and P=0.079). The difference in walking, running, sitting and sleeping behaviour of male and female ostriches were not significant (P > 0.05). The standing and sitting behaviours of ostriches were affected by time of day (P=0.49 and P=0.026, respectively). The lowest standing and highest sitting behaviour were observed at noon and evening. The walking, running, alert and sleeping behaviour of ostriches were not changed during the day (P > 0.05). The effect of enriched environment and gender interaction on standing behaviour of ostriches was found significant (P = 0.031), lowest standing behaviour was found in females in enrichment group. The effect of enriched environment and gender interaction on walking, running, alert, sitting and sleeping behaviours of ostriches were not significant (P > 0.05). The effect of enriched environment and time of day interaction on standing behaviour of ostriches was found significant (P =0.023) and lowest standing behaviour was observed in gravel group during the evening. The three-way interaction effect of enriched environment, gender and time of day on locomotor and resting behaviours of ostrich was not significant (P > 0.05).

The effects of enriched environment with natural vegetation on comfort behaviours of ostriches were given in Table 3. The effect of enriched environment on dust bathing, preening, head shake and thermoregulation behaviours of ostrich was not significant (P > 0.05). The gender of ostrich effected dust bathing behaviour, and only females showed dustbathing behaviour during to study (P=0.040). The difference in preening, head shake and thermoregulation behaviour of male and female ostriches were not significant (P > 0.05). The dustbathing behaviour was observed only during the evening (P=0.018). The preening, head shake and thermoregulation behaviour of ostriches were not changed during the day (P > 0.05). The effect of enriched environment and gender interaction; and enriched environment and time of day interaction on comfort behaviours of ostriches were not significant (P > 0.05). The three-way interaction effect of enriched environment, gender and time of day on comfort behaviours of ostrich was not significant (P > 0.05);

Table 2. Effects of enriched environment on locomotor and resting behaviours of ostriches (number of bouts/hour)

Enrichment		Walking	Running	Alert	Standing	Sitting	Sleeping
Floor	Gravel	0.13 ± 0.02	0.003± 0.003	0.027 ± 0.01	0.10 ± 0.01	0.083 ± 0.01	0.018±0.01
	Vegetation	0.11 ± 0.02	0.006± 0.003	0.022 ± 0.01	0.09 ± 0.01	0.041 ± 0.01	0.006±0.01
P		*0.460*	*0.468*	*0.709*	*0.493*	*0.109*	*0.290*
Gender	Male	0.12 ± 0.02	0.006± 0.003	0.039 ± 0.01	0.11 ± 0.01	0.083 ± 0.01	0.014±0.01
	Female	0.11 ± 0.02	0.003± 0.003	0.010 ± 0.01	0.08 ± 0.01	0.041 ± 0.01	0.010±0.01
P		*0.854*	*0.611*	*0.059*	*0.079*	*0.101*	*0.707*
Time	MO	0.10 ± 0.02	0.003± 0.004	0.037 ± 0.01	0.12 ± 0.02^{a}	0.015 ± 0.02^{b}	0.007±0.01
of Day	NO	0.12 ± 0.02	0.011± 0.004	0.026 ± 0.01	0.10 ± 0.02^{ab}	0.103 ± 0.02^{a}	0.025±0.01
	EV	0.14 ± 0.02	-	0.011 ± 0.01	0.07 ± 0.02^{b}	0.069 ± 0.02^{ab}	0.004±0.01
P		*0.388*	*0.134*	*0.372*	*0.049*	*0.026*	*0.250*
Floor X Gender							
G X Male		0.13 ± 0.03	0.006± 0.004	0.044 ± 0.02	0.10 ± 0.02^{ab}	0.117 ± 0.03	0.022±0.01
G X Female		0.12 ± 0.03	-	0.010 ± 0.02	0.11 ± 0.02^{a}	0.049 ± 0.03	0.013±0.01
V X Male		0.11 ± 0.03	0.006± 0.004	0.033 ± 0.02	0.13 ± 0.02^{a}	0.050 ± 0.03	0.006±0.01
V X Female		0.11 ± 0.03	0.007± 0.004	0.010 ± 0.02	0.06 ± 0.02^{b}	0.033 ± 0.03	0.007±0.01
P		*0.789*	*0.468*	*0.709*	*0.031*	*0.324*	*0.641*
Floor X Time of day							
G X MO		0.11 ± 0.03	-	0.030 ± 0.02	0.16 ± 0.02^{a}	0.030 ± 0.03	-
G X NO		0.10 ± 0.03	0.008± 0.005	0.030 ± 0.02	0.11 ± 0.02^{ab}	0.143 ± 0.03	0.045±0.01
G X EV		0.17 ± 0.03	-	0.022 ± 0.02	0.04 ± 0.02^{c}	0.076 ± 0.03	0.008±0.01
V X MO		0.08 ± 0.03	0.004± 0.005	0.04 3± 0.02	0.09 ± 0.02^{bc}	-	0.013±0.01
V X NO		0.13 ± 0.03	0.013± 0.005	0.02 2± 0.02	0.09 ± 0.02^{bc}	0.063 ± 0.03	0.005±0.01
V X EV		0.11 ± 0.03	-	-	0.10 ± 0.02^{bc}	0.061 ± 0.03	-
P		*0.399*	*0.874*	*0.628*	*0.023*	*0.547*	*0.149*
FloorX Gender X Time of Day							
P		*0.385*	*0.630*	*0.924*	*0.474*	*0.595*	*0.985*

^{a,b,c}: The different superscripts on numbers represent a significant difference between them (*P*<0.05).
Data are presented as LSM ± SEM.
G: Gravel, V: Vegetation, MO: Morning, NO: Noon, EV: Evening, - : No Behaviour

except for dust bathing behaviour (P=0.038).

The effects of enriched environment with natural vegetation on reproduction behaviours of ostriches were given in Table 4. The boom and courtship behaviours were affected by enrichment, and higher boom and courtship behaviour were observed in enrichment group (P=0.050 and P=0.028, respectively). However, the effect of enriched environment on kantel, mating and laying behaviours were similar (P > 0.05). Only male ostrich showed boom behaviour (P=0.050) and only females were showed laying behaviour (P=0.025). The courtship, kantel and mating behaviours did not change by the gender of ostrich (P > 0.05). The effect of time of day on reproduction behaviours of ostriches were found not significant (P > 0.05); except for laying behaviour (P=0.009). Laying was observed only evening during to study (P=0.009). The effect of enriched environment and gender interaction; and enriched environment and time of day interaction on reproduction behaviours of ostriches were not significant (P > 0.05). The three-way interaction effect of enriched environment, gender and time of day on reproduction behaviours of ostrich was not significant (P > 0.05); except for laying behaviour (P=0.012).

The effects of enriched environment with natural vegetation on aggressive behaviours of ostrich were given in Table 5. The effect of enriched environment, gender and time of day on threat, fight and escape behaviours of ostriches were not significant (P > 0.05). The effect of enriched environment and gender interaction; and enriched environment and time of day interaction on aggressive behaviours of ostriches were not significant (P > 0.05). However, displace behaviour was only observed in females in enriched with vegetation group (P= 0.001). The three-way interaction effect of enriched environment, gender and time of day on aggressive behaviours of ostrich was not significant (P > 0.05); except for escape and threat behaviours (P=0.044 and P=0.064).

The three-way interaction effect of enriched environment, gender and time of day on ostrich behaviours were given in Table 6. The dust bathing, laying and escape behaviours of ostriches were affected by enriched environment, gender and time of day interaction (P=0.038, P=0.012, and P=0.044, respectively). And the effect of triple interaction on threat behaviour tends to be significant (P=0.064). There was a high frequency of dust bathing and laying behaviour in both floor group for females during the evening hours. There was a high frequency of escape behaviour for females during to evening hours in enriched group. The highest threat behaviour frequency was observed for males during to morning hours in control group.

Table 3. Effects of enriched environment on comfort behaviours of ostriches (number of bouts/hour)

Enrichment		Dust Bathing	Preening	Head Shake	Thermoregulation
Floor	Gravel	0.005 ± 0.00	0.07 ± 0.01	0.007 ± 0.01	0.06 ± 0.02
	Vegetation	0.005 ± 0.00	0.04 ± 0.01	0.016 ± 0.01	0.05 ± 0.02
		1.000	*0.259*	*0.220*	*0.564*
Gender	Male	-	0.05 ± 0.01	0.017 ± 0.01	0.05 ± 0.02
	Female	0.010 ± 0.00	0.06 ± 0.01	0.007 ± 0.01	0.06 ± 0.02
		0.040	*0.884*	*0.161*	*0.929*
Time	MO	-	0.05 ± 0.02	0.013 ± 0.01	0.02 ± 0.03
of Day	NO	-	0.07 ± 0.02	0.012 ± 0.01	0.08 ± 0.03
	EV	0.015 ± 0.00	0.04 ± 0.02	0.011 ± 0.01	0.06 ± 0.03
P		*0.018*	*0.397*	*0.980*	*0.317*
Floor X Gender					
G X Male		-	0.08 ± 0.02	0.011 ± 0.01	0.06 ± 0.03
G X Female		0.010 ± 0.01	0.05 ± 0.02	0.003 ± 0.01	0.07 ± 0.03
V X Male		-	0.03 ± 0.02	0.022 ± 0.01	0.04 ± 0.03
V X Female		0.010 ± 0.01	0.06 ± 0.02	0.010 ± 0.01	0.05 ± 0.03
P		*1.000*	*0.145*	*0.748*	*0.963*
Floor X Time of Day					
G X MO		-	0.07 ± 0.02	0.008 ± 0.01	0.04 ± 0.04
G X NO		-	0.09 ± 0.02	-	0.05 ± 0.04
G X EV		0.015 ± 0.01	0.03 ± 0.02	0.013 ± 0.01	0.10 ± 0.04
V X MO		-	0.02 ± 0.02	0.017 ± 0.01	0.01 ± 0.04
V X NO		-	0.06 ± 0.02	0.023 ± 0.01	0.11 ± 0.04
V X EV		0.015 ± 0.01	0.05 ± 0.02	0.008 ± 0.01	0.02 ± 0.04
P		*1.000*	*0.269*	*0.285*	*0.224*
FloorX Gender X Time of Day					
P		*0.038*	*0.796*	*0.407*	*0.963*

Data are presented as LSM ± SEM.
G: Gravel, V: Vegetation, MO: Morning, NO: Noon, EV: Evening, - : No Behaviour

Table 4. Effects of enriched environment on reproduction behaviours of ostriches (number of bouts/hour)

Enrichment		Boom	Courtship	Kantel	Mating	Laying
Floor	Gravel	-	0.010 ± 0.01[b]	0.022 ± 0.01	0.009 ± 0.00	0.003 ± 0.00
	Vegetation	0.008 ± 0.00	0.036 ± 0.01[a]	0.013 ± 0.01	0.009 ± 0.00	0.003 ± 0.00
P		*0.050*	*0.028*	*0.462*	*1.000*	*1.000*
Gender	Male	0.008 ± 0.00	0.025 ± 0.01	0.025 ± 0.01	0.011 ± 0.00	-
	Female	-	0.021 ± 0.01	0.010 ± 0.01	0.007 ± 0.00	0.007 ± 0.00
P		*0.050*	*0.743*	*0.185*	*0.452*	*0.025*
Time	MO	0.008 ± 0.00	0.009 ± 0.01	0.011 ± 0.01	0.007 ± 0.01	-
of Day	NO	0.004 ± 0.00	0.022 ± 0.01	0.035 ± 0.01	0.007 ± 0.01	-
	EV	-	0.038 ± 0.01	0.007 ± 0.01	0.013 ± 0.01	0.010 ± 0.00
P		*0.263*	*0.133*	*0.103*	*0.588*	*0.009*
Floor X Gender						
G X Male		-	0.017 ± 0.01	0.033 ± 0.01	0.011 ± 0.01	-
G X Female		-	0.003 ± 0.01	0.010 ± 0.01	0.007 ± 0.01	0.007 ± 0.00
V X Male		0.017 ± 0.00	0.033 ± 0.01	0.017 ± 0.01	0.011 ± 0.01	-
V X Female		-	0.039 ± 0.01	0.010 ± 0.01	0.007 ± 0.01	0.007 ± 0.00
P		*0.050*	*0.403*	*0.462*	*1.000*	*1.000*
Floor X Time of day						
G X MO		-	0.013 ± 0.01	0.022 ± 0.01	-	-
G X NO		-	-	0.030 ± 0.01	0.013 ± 0.01	-
G X EV		-	0.017 ± 0.01	0.013 ± 0.01	0.013 ± 0.01	0.10 ± 0.00
V X MO		0.017 ± 0.01	0.005 ± 0.01	-	0.013 ± 0.01	-
V X NO		0.008 ± 0.01	0.045 ± 0.01	0.040 ± 0.01	-	-
V X EV		-	0.059 ± 0.01	-	0.013 ± 0.01	0.010 ± 0.00
P		*0.263*	*0.116*	*0.503*	*0.215*	*1.000*

FloorX Gender X Time of Day						
P		*0.233*	*0.865*	*0.907*	*0.992*	*0.012*

[a,b]: The different superscripts on numbers represent a significant difference between them (*P*<0.05).
Data are presented as LSM ± SEM.
G: Gravel, V: Vegetation, MO: Morning, NO: Noon, EV: Evening, - : No Behaviour

Table 5. Effects of enriched environment on aggressive behaviours of ostriches (number of bouts/hour)

Enrichment		**Threat**	**Fight**	**Escape**	**Displace**
Floor	Gravel	0.016 ± 0.01	0.011 ± 0.01	-	-
	Vegetation	0.006 ± 0.01	-	0.008 ± 0.00	0.016 ± 0.00
P		*0.277*	*0.153*	*0.097*	*0.001*
Gender	Male	0.017 ± 0.01	0.011 ± 0.01	0.003 ± 0.00	-
	Female	0.005 ± 0.01	-	0.005 ± 0.00	0.016 ± 0.00
P		*0.179*	*0.153*	*0.638*	*0.001*
Time of Day	MO	0.017 ± 0.01	0.008 ± 0.01	-	0.005 ± 0.00
	NO	0.007 ± 0.01	-	0.004 ± 0.00	0.007 ± 0.00
	EV	0.009 ± 0.01	0.008 ± 0.01	0.007 ± 0.00	0.012 ± 0.00
P		*0.611*	*0.590*	*0.415*	*0.422*
Floor X Gender					
G X Male		0.028 ± 0.01	0.022 ± 0.01	0.006 ± 0.01	-
G X Female		0.003 ± 0.01	-	0.010 ± 0.01	-
V X Male		0.006 ± 0.01	-	-	-
V X Female		0.007 ± 0.01	-	-	0.033 ± 0.01
P		*0.147*	*0.153*	*0.638*	*0.001*
Floor X Time of day					
G X MO		0.033 ± 0.01	0.017 ± 0.01	-	-
G X NO		0.005 ± 0.01	-	-	-
G X EV		0.008 ± 0.01	0.017 ± 0.01	-	-
V X MO		-	-	-	0.010 ± 0.01
V X NO		0.008 ± 0.01	-	0.008 ± 0.01	0.015 ± 0.01
V X EV		0.010 ± 0.01	-	0.015 ± 0.01	0.025 ± 0.01
P		*0.161*	*0.590*	*0.415*	*0.422*
FloorX Gender X Time of Day					
P		*0.064*	*0.736*	*0.044*	*0.493*

Data are presented as LSM ± SEM.
G: Gravel, V: Vegetation, MO: Morning, NO: Noon, EV: Evening, - : No Behaviour

Table 6. The three-way interaction effect enriched environment, gender and time of day on ostrich behaviours (number of bouts/hour)

Floor X Gender X Time of Day	**Dust Bathing**	**Laying**	**Threat**	**Escape**
G X M X MO	0.000 ± 0.01[b]	0.000 ± 0.00[b]	0.067 ± 0.01[a]	0.000 ± 0.01[c]
G X M X NO	0.000 ± 0.01[b]	0.000 ± 0.00[b]	0.000 ± 0.01[c]	0.000 ± 0.01[c]
G X M X EV	0.000 ± 0.01[b]	0.000 ± 0.00[b]	0.017 ± 0.01[b]	0.000 ± 0.01[c]
G X F X MO	0.000 ± 0.01[b]	0.000 ± 0.00[b]	0.000 ± 0.01[c]	0.000 ± 0.01[c]
G X F X NO	0.000 ± 0.01[b]	0.000 ± 0.00[b]	0.010 ± 0.01[b]	0.000 ± 0.01[c]
G X F X EV	0.029 ± 0.01[a]	0.020 ± 0.00[a]	0.000 ± 0.01[c]	0.000 ± 0.01[c]
V X M X MO	0.000 ± 0.01[b]	0.000 ± 0.00[b]	0.000 ± 0.01[c]	0.000 ± 0.01[c]
V X M X NO	0.000 ± 0.01[b]	0.000 ± 0.00[b]	0.017 ± 0.01[b]	0.017 ± 0.01[b]
V X M X EV	0.000 ± 0.01[b]	0.000 ± 0.00[b]	0.000 ± 0.01[c]	0.000 ± 0.01[c]
V X F X MO	0.000 ± 0.01[b]	0.000 ± 0.00[b]	0.000 ± 0.01[c]	0.000 ± 0.01[c]
V X F X NO	0.000 ± 0.01[b]	0.000 ± 0.00[b]	0.000 ± 0.01[c]	0.000 ± 0.01[c]
V X F X EV	0.029 ± 0.01[a]	0.020 ± 0.00[a]	0.020 ± 0.01[b]	0.029 ± 0.01[a]
P	*0.038*	*0.012*	*0.064*	*0.044*

[a,b]: The different superscripts on numbers represent a significant difference between them (*P*<0.05).
Data are presented as LSM ± SEM.
G: Gravel, V: Vegetation, M: Male, F: Female; MO: Morning, NO: Noon, EV: Evening, - : No Behaviour.

Discussion

In the study ostriches spent more time with eating, foraging, walking and standing in all investigated behaviours. Thus, these findings are corroborated by earlier researches from Ahmed & Salih (2012) and Mutiga et al. (2016).

The physical components of the enclosure design (such as shelters, plants, rocks, and pools), transient objects (such as food items, balls, and branches), and non-object stimuli (such as sounds, climatic and lighting variables) can all be used to enrichment of captive birds. All of these categories have the potential to elicit actions brought on by the stimulation of one or more senses, and their combination can satisfy welfare-related requirements (King, 1999). There were some studies about environmental enrichment with vegetation on behaviours of poultry. Thus, in their environmental enrichment study; Stadig et al. (2017) found that Sasso T451 broilers showed generally higher activity levels and use of the area with dense vegetation (short rotation coppice) compared to grassland with artificial shelters. When Dal Bosco et al. (2014) compared the ecologically enriched range (with sorghum grass strips or mature olive trees) to the control range, they reported that enrichment encouraged free-range broilers to go outside. Also, Dawkins et al. (2003) discovered that the number of Sherwood Whites birds ranging outside was positively connected with the amount of grass, trees and bushes cover offered.

The mature tree cover in free range system effected the behaviours of the female Ross 308 broilers (Jones et al., 2007). Ostriches in the wild eat grass, berries, succulents, seeds, and tree and shrubs' leaves (Sambraus, 1994; Deeming & Bubier, 1999). Thus, access to outdoor spaces with different types of nutritious vegetation (grass/clover or chicory) boosted foraging activity in broilers, according to Almeida et al. (2012). However, in our study, the environmental enrichment with vegetation did not affect eating, drinking, foraging, pecking and defecation behaviour of ostriches. This might be due to the use of natural vegetation for environmental enrichment that did not have much nutritional value. Similar to our findings, Carvalho et al. (2017) reported there were no differences on feeding and biting behaviour of cockatiels in captivity when collard green stalks used as environmental enrichment.

The gender of ostrich effected foraging behaviour, and higher foraging behaviour was observed in females. Similar to our findings Deeming (1998) reported that females had higher foraging behaviour than males. However, there was no gender difference on eating, drinking, pecking and defecation behaviour of ostriches. These findings supported by Bertram (1992) and Mutiga et al. (2016) who found that gender did not affect feeding behaviour time budget in ostriches.

The time of day affected eating and foraging behaviours, and not affected pecking and defecation behaviours of ostriches. The higher eating behaviour was observed in the morning and evening, and the highest foraging behaviour was observed during morning. Thus, Amado et al. (2011), Ahmed & Salih (2012) and Mutiga et al. (2016) found that feed consumption was higher in the morning and in the afternoon. In reality, birds may restrict their feed consumption in order to avoid the internal heat rise caused by digestion during the warmer hours of the day. The drinking behaviour tends to be higher in the morning and at noon. But Amado et al. (2011) and Ahmed & Salih (2012) reported that drinking behaviour was higher in the afternoon.

Food as environmental enrichment should be encouraged due to its favorable impacts on animal welfare; substantial variations in walking, foraging, eating feces, and pacing behaviours of male Greater rheas in zoo with enrichment with fruits were identified (Lima et al. 2019). Christensen and Nielsen (2004) used sand-covered areas with barren or enriched with cabbage, coniferous cones, and sticks and they reported that environmental enrichment improves the welfare of chicks by increasing exploration and decreasing pecking without compromising food consumption in commercially reared ostrich chicks. However, in the study lowest eating behaviour was found in males in enrichment group. But there was no interaction between enrichment and gender on drinking, foraging, pecking and defecation behaviours of ostriches. Also, there was no interaction between enrichment and time of day on ingestive behaviours of ostriches; except for pecking behaviour. The higher pecking behaviour was observed in the evening in gravel group and in morning in enrichment group.

Lubac & Mirabito (2001) and Mirabito et al. (2001) found that shaded regions under established trees prompted the broilers to lie down, whereas standing was the prevalent behaviour in non-shaded areas. Also, according to Csermely et al. (2007), ostriches kept in captivity exhibit stood-still behaviour more often because of frustration or a constrained environment. Another study, Carvalho et al. (2017) reported that collard green stalks when used as environmental enrichment, decreased sleep behaviour in cockatiels at captivity. However, in the study, the environmental enrichment with vegetation did not affect walking, running, alert, standing, sitting, sleeping behaviour of ostriches. This may be because the natural vegetation used for environmental enrichment did not contain plants or trees that were too tall or large to hinder the movements of birds. Similar to our results, Carvalho et al. (2017) also reported that collard green stalks were used as environmental enrichment of cockatiels in captivity, but its use did not significantly affect locomotion and resting behaviour of birds.

The gender of ostrich tends to be affected alert and standing behaviour, and higher alert and standing behaviour was observed in males. This could be because

females were always busy while performing behaviours like foraging. Thus, males exhibited a proportionally higher percentage of time in the alert state than females, according to Mutiga et al. (2016). In the study there was no gender difference on walking, running, sitting and sleeping behaviour of ostriches. But males spent more time with resting than females, according to Mutiga et al. (2016).

The time of day affected standing and sitting behaviours, and was not affected walking, running, alert and sleeping behaviours of ostriches. The lowest standing and highest sitting behaviour were observed at noon and evening. There were some studies reported that ostriches stand still more in the morning (Amado et al., 2011; Ahmed & Salih, 2012). Also, in accordance with our findings Mutiga et al. (2016) discovered that daytime had no effect on alertness behaviour of ostriches.

The lowest standing behaviour was found in females in enrichment group. However, there was no interaction between enrichment and gender on walking, running, alert, sitting and sleeping behaviours of ostriches. Also, there was no interaction between enrichment and time of day on walking, running, alert, sitting and sleeping behaviours of ostriches; except for standing behaviour. The lowest standing behaviour was observed in gravel group during the evening.

For thermoregulation, ostriches tend to breathe frequently (panting) to cope with the heat during hot hours (Maloney, 2008). In the study, the environmental enrichment with vegetation did not affect dust bathing, preening, head shake and thermoregulation behaviour of ostriches. In accordance with our results, Carvalho et al. (2017) reported that collard green stalks were used as environmental enrichment of cockatiels in captivity, but its use did not significantly affect body surface temperature and maintenance behaviour of birds.

Dust bathing and preening is a crucial behaviour for ostriches to maintain optimum feather health. The gender of ostrich effected dust bathing behaviour, and only females showed dustbathing behaviour during to study. However, there was no gender difference on preening, head shake and thermoregulation behaviour of ostriches. But males spend more time preening than females, according to Mutiga et al. (2016).

The time of day effected dustbathing behaviours, and was not affected preening, head shake and thermoregulation behaviours of ostriches. However, ostriches preening more frequently in the morning than in the afternoon (Deeming & Bubier, 1999). The dustbathing behaviour was observed only in the evening. Our results were similar with Amado et al. (2011) and Ahmed & Salih (2012) who found that dust bathing was observed generally late hours of the day. High temperatures during the day may have contributed to a decrease in birds' activity. There was no interaction between enrichment and gender; and also enrichment and time of day on comfort behaviours of ostriches.

In our study, the environmental enrichment with vegetation effected boom and courtship behaviour of ostriches and higher boom and courtship behaviour were observed in the enrichment group. Thus, Cooper et al. (2010) show that ostriches are opportunistic breeders whose reproduction is reliant on the quality and quantity of feed. However, the environmental enrichment with vegetation did not affect kantel, mating and laying behaviours of ostriches.

The gender of ostrich affected boom and laying behaviour. Thus, according to Aravinth & Selvan (2015) and Mukhtar et al. (2017) rhythmic booming sound signals the onset of mating in the male and helps attract female attention for courtship. Additionally, males do the "kantel" breeding dance to attract females for reproduction in ostriches. However, there was no gender difference on courtship, kantel and mating behaviours of ostriches.

Mating activity in ostriches was seen throughout the morning hours, according to Sambraus (1994). But, in our study, time of day was only affected laying behaviour among all of the reproduction behaviours. Laying was observed only in the evening during study. Supporting our findings Brassó et al. (2020) reported that ostrich eggs were typically laid in the afternoon or early evening. There was no interaction between enrichment and gender; and also enrichment and time of day on reproduction behaviours of ostriches.

The use of strong methods like environmental enrichment, imprinting, foster parenting, and regular handling can help reduce stress and address many of the constraints put on behaviour due to stress in domestic chicks (Jones & Waddington, 1993). However, in the study, the environmental enrichment with vegetation effected displace behaviour, but enrichment did not affect threat, fight and escape behaviour of ostriches.

The gender of ostrich affected displace behaviour, but did not affect threat, fight and escape behaviour of ostriches. The time of day did not affect aggressive behaviours of ostriches. However, according to Fericean et al. (2022), aggressive behaviours were higher in the morning than in the afternoon and at night. There was no interaction between enrichment and gender; and also, enrichment and time of day on aggressive behaviours of ostriches; except for displace behaviour. The displace behaviour was only observed in females in the enriched with vegetation group.

Conclusion

Due to the stress caused by adverse environmental conditions, ostriches grown on crowded farms begin to exhibit abnormal behaviours (Şahan et al., 2000). Thus, Kock (1996a, 1996b), giving captive ostriches access to a more natural habitat appears to reduce their stress and agitation. Also, by enabling animals to express more of their species-specific behavioural repertoire and to accommodate a wider range of behavioural options, enriched environments

can improve the welfare of animals (Van de Weerd & Day, 2009).

As a conclusion, ostriches are kept in paddocks in open areas in intensive farming systems. Vegetation growing in the paddocks is routinely cleaned and production is carried out in an environment different from bird's natural environment. Environmental enrichment with natural vegetation in paddock only affected ostriches' courtship behaviours, also effected male and female eating and standing behaviours differently. Through behavioural research, it is possible to identify animals' stressful conditions and enhance their wellbeing. Regarding environmental enhancement and welfare in ostrich welfare issues, there is much to learn.

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RESEARCH PAPER

Influence of Phytobiotics and Organic Acid on Nutrient Utilization Efficiency and Carcass Characteristics of Broiler

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Abstract

A total of 120-day old straight run broiler chicks (cobb 500) were randomly assigned into four groups having three replications in each. In control group (To) only mash feed (ME- 3028 kcal/kg, CP- 22.72%) was supplied and in other groups (TN, TM, TC), control + 1% dried Neem leaf powder, Control + 1% dried Moringa leaf powder and control + 1% Citric acid were supplied respectively. The entire period for feeding trial was 28 days. At last day of the feeding trial blood and meat samples were collected. The body weight gain of broilers was observed as the highest in Tc group ($p<0.05$). Feed conversion ratio (FCR) and Metabolizable energy (ME) intake per gram gain (4.63) was lower ($p < 0.05$) in TM than the other groups of birds. Energy efficiency ratio in TM and protein efficiency ratio in TN was higher ($p < 0.05$) than other groups of birds. CP content was high ($p < 0.05$) in TN. Drip loss and cooking loss was lower ($p < 0.05$) in TM than other groups. Based on the findings of the present experiment, it could be concluded that, supplementation of dried Moringa leaf powder has stimulating effect on nutrient utilization and carcass characteristics of broiler and useful phytobiotics for safe and high-quality broiler meat production.

Introduction

The increasing trends of microbial resistance and accumulation of residues in broiler meat due to the use of synthetic antibiotic growth promoters in broiler feed has a bad effect on human health. Broiler producers of most low and middle-income countries use different types of antibiotic growth promoter indiscriminately, which also hampers animal welfare. But it is very important to increase the production of animal protein like broiler to cope with a huge demand. Moreover, producers have become more interested in producing broiler meat using antibiotic growth promoters, as they enhance the production rate as well as reduce the production cost. Nowadays consumers are more interested in safe broiler meat and the market for safe broiler is increasing day by day. So, it is very important to search a new alternative to synthetic antibiotic growth promoters and in that situation, phytogenic feed additive could be one of the best solutions (Ghalamkari *et al.,* 2012; David *et al.,* 2016). Moreover, phytogenic feed additives are non-toxic, natural, free from residual effects (El-Hack *et al.,* 2020; Magdalena *et al.,* 2021) and also enhance digestibility by stimulating the digestive enzyme secretion, boosting up immunity as well as show antiviral effects (Oh *et al.,* 2013; Ognik *et al.,* 2020). Non-nutritive feed additives like antibiotics used in sub therapeutic level in animal production create antimicrobial resistance in human due to the production of residues in animal products (Durrani *et al.,* 2008). Medicinal herb enhances the feed quality and maximizes carcass output in broiler (Ali *et al.,* 2019). Different types of phytobiotics have gained popularity nowadays.

Neem is one the best phytobiotics and many parts of this plants is used for medication. Neem leaves have quercetin, nimbosterol, liminoids which enhance broiler growth as well as feed utilization efficiency

(Prasannabalaji, 2012). Moringa leaves are also responsible for better growth performance of broiler as well as have some effects on meat color and quality (Naji, 2013) which is one of most consumers` preferences. Organic acid like citric acid exhibit similar effects of phytobiotics in broiler (Khan *et al.,* 2016) and enhances broiler performance through modulating gut homeostasis (Sabour, 2019). So, the present experiment was conducted to explore the effects of medicinal herb and organic acid on nutrient utilization efficiency and carcass characteristics of broiler.

Materials and Methods

Feeding trial

Feeding trial was conducted in Shahjalal animal Nutrition Field laboratory, Bangladesh Agricultural University, Bangladesh. A total of 120-day old chicks (cobb 500) were purchased from local distributor and chicks were randomly distributed into four groups having three replications in each group (10 chicks in each replication). Mash feed (Table 1) was offered to chicks which was prepared following NRC (1994).

Table 1: Composition of ration for different dietary group

Ingredient	Amount (%)
Maize	46.50
Protein concentrate	8.50
Rice Polish	10.00
Soybean Meal	29.00
DCP	1.50
Soybean Oil	3.00
Salt	0.50
Lysine	0.25
DL-Methionine	0.25
Vit-Mineral premix	0.50
Total	100.00
Nutrient Composition (Calculated)	
ME (Kcal/kg)	3028
CP (%)	22.72
CF (%)	4.85
Ca (%)	0.68
P (%)	0.31

Vitamin-mineral premix composition: Vitamin A 12,500,000 IU, Vitamin D_3 2,500,000 IU, Vitamin E 20,000 mg, Vitamin K_3 4,000 mg, Iron 40,000 mg Vitamin B_1 2,500 mg, Vitamin B_2 5,000 mg, Vitamin B_6 4,000 mg Nicotinic acid 40,000 mcg, Pantothenic acid 12,500 mg, Vitamin B_{12} 12,000 mcg, Folic acid 800 mg, Biotin 100 mg, Cobalt 400 mg, Copper 10,000 mg, Iodine 400 mg, Manganese 60,000 mg, Zinc 50,000 mg, Selenium 150 mg, Di-Calcium Phosphate 380 gm

The feed for all groups of birds were iso caloric and iso nitrogenous. Moringa (*Moringa olifera*), Neem (*Azadiracta indica*) dried leaf powder and citric acid were purchased locally. In Control group (T_o) only the mash feed was supplied and in other groups, control + 1% dried Neem leaf powder, Control + 1% dried Moringa leaf powder and control + 1% ascorbic acid were supplied in T_N, T_M, T_C group respectively. Birds of all replications were reared in separate pens and that was assigned unbiasedly. Saw dust were used as litter material. Feeding trial was conducted for 28 days and feed and water was supplied ad-libitum throughout the feeding trial. At the age of 4th, 11th and 19th days, ND, IBD and ND booster vaccines were provided through eye. Throughout the feeding trial period, body weight and feed intake were collected on weekly basis.

Collection of samples and analysis

At 29th day of the feeding trial blood samples (5 ml) were collected from unbiasedly selected birds from each replication and centrifuged at 6000 rpm for 10 minutes for serum separation. Separated serum were stored at -20^o C for further analysis of blood metabolites. Then birds were slaughtered and collected meat samples from breast and thigh and preserved the last day of the feeding trial. The parameters include total protein, albumin, globulin, creatinine, urea, calcium, phosphorus, total cholesterol, HDL and LDL in blood were analyzed using commercially available kits following the method of kit manufacturer. Proximate analysis of thigh and breast meat were performed following the method established by AOAC (1995). p^H of meat was measured within 30 minutes after slaughter (initial p^H) and after 24 hours of storage (p^Hu). Moreover, energy efficiency ratio was calculated according to Kamran (2008) as gram of weight gain× 100/total ME intake and protein efficiency ratio was measured following the formula of McDonald (1995).

Statistical analysis

All data were subjected to analysis of variance (ANOVA) in a Completely Randomized Design (CRD) to test the significance of treatment effects and comparison of treatment mean was performed using Tukey's HSD test. SPSS statistical analysis software (SPSS Inc. Chicago, IL, USA) was used for all statistical analysis. Comparison of means was carried out at 5% level of significance ($p < 0.05$).

Results

Nutrient Utilization

Weight gain, FCR, Protein efficiency ratio, Energy efficiency ratio, ME intake per gram gain and CP intake per gram gain was significantly affected in the present experiment. Weight gain was high in T_C group ($p < 0.05$). FCR (1.60) and ME intake per gram gain (4.63) was lower ($p < 0.05$) in T_M group than the other groups of birds (Table 2). Energy efficiency ratio (22.13) in T_M group and Protein efficiency ratio (2.80) in T_N group was higher ($p < 0.05$) than other groups of birds.

Meat quality

In case of breast muscle, significant difference was found in dry matter, crude protein, drip loss and

cooking loss (Table 3). Dry matter was significantly high (29.02%) in neem leaf supplemented groups (T_N). concentration of glucose in blood was high ($p < 0.05$) in control group of birds than others.

Table 2: Growth performance of birds

Parameter	T_0	T_N	T_M	T_C	*p*-value
Body Weight gain	1371^{b}±15	1326^{c}±25	1390^{b}±29	1430^{a}±35	0.021
Feed intake	2413±40	2150±35	2224±28	2384±32	0.210
FCR	1.76^{a}±0.11	1.62^{b}±0.15	1.60^{b}±0.11	1.66^{ab}±0.14	0.014
Protein efficiency ratio	2.55^{b}±0.32	2.80^{a}±0.12	2.78^{ab}±0.18	2.73^{ab}±0.22	0.001
Energy efficiency ratio	18.13^{c}±2.12	19.26^{a}±3.02	22.13^{ab}±2.52	20.68^{b}±2.31	0.033
ME intake per gram gain (kj/g)	5.27^{a}±0.24	4.70^{b}±0.36	4.63^{b}±0.39	4.84^{ab}±0.11	0.009
CP intake per gram gain (g)	0.43^{a}±0.05	0.36^{b}±0.03	0.35^{b}±0.6	0.37^{ab}±0.9	0.019

T_0 = Control group, T_N = Control+ 1% dried Neem leaf powder, T_M = Control+ 1% dried Moringa leaf powder, T_C = Control + 1% Citric acid

^{abc} means bearing dissimilar superscript in same row differ significantly at the level of 5%

Among the four groups of birds, crude protein content was high ($p < 0.05$) in T_N. Like breast muscle, dry matter and crude protein content was high in T_N groups of birds. But ether extract content was significantly lower in citric acid supplemented group of birds in both breast and thigh muscle. Drip loss and cooking loss was lower ($p < 0.05$) in T_M than other groups of birds.

Discussion

Nutrient Utilization Efficiency

Medicinal plant has beneficial effects on broiler growth performance. In this experiment, weight gain of broiler was high in T_C. Demirel (2012) stated that supplementation of citric acid had increased body

Table 3: Carcass characteristics

	T_0	T_N	T_M	T_C	*p*-value
Breast muscle:					
P^H (Initial)	6.20±0.63	6.05±0.32	5.70±0.65	5.78±0.45	0.342
P^Hu (after 24 h)	5.80±0.51	5.97±0.25	5.71±0.33	5.70±0.21	0.411
Dry matter (%)	27.75^{b}±2.2	29.02^{a}±2.4	27.02^{c}±1.89	26.30^{d}±1.7	0.023
Crude protein (% DM)	25.22^{b}±2.4	26.51^{a}±2.4	24.98^{c}±1.5	24.55^{c}±1.69	0.002
Ether extract (% DM)	1.22^{a}±0.12	0.95^{c}±0.09	1.01^{c}±0.05	1.12^{b}±0.02	0.030
Ash (%DM)	1.08±0.12	1.19±0.32	1.15±0.09	1.14±0.08	0.532
Water holding capacity	57±4.03	66±5.12	68±7.01	65±3.22	0.222
Drip loss	15^{a}±2.12	13^{b}±1.65	9^{ab}±2.03	11^{ab}±3.11	0.011
Cooking loss	33^{a}±3.05	32^{ab}±5.18	28^{b}±2.12	30^{b}±2.03	0.001
Thigh muscle:					
P^H (Initial)	5.99±0.53	5.87±0.23	5.18±0.48	5.43±0.54	0.443
P^Hu (after 24 h)	5.70±0.16	5.81±0.31	5.17±0.29	5.33±0.25	0.087
Dry matter (%)	23.56^{c}±2.6	24.22^{a}±1.5	23.86^{b}±1.8	24.03^{ab}±1.7	0.002
Crude protein (% DM)	20.76^{b}±1.8	21.89^{a}±1.6	21.07^{ab}±1.5	21.45^{a}±1.32	0.034
Ether extract (% DM)	1.77^{a}±0.12	1.51^{b}±0.09	1.68^{a}±0.3	1.50^{b}±0.08	0.003
Ash (%DM)	0.95±0.01	1.05±0.08	1.12±0.08	1.19±0.06	0.731
Water holding capacity	59±3.07	63±6.09	65±4.11	61±2.04	0.882
Drip loss	13^{a}±2.10	11^{b}±1.01	10^{ab}±1.20	14^{a}±1.80	0.003
Cooking loss	36^{a}±3.05	30^{ab}±5.04	26^{b}±2.15	33^{b}±2.07	0.047

T_0 = Control group, T_N = Control+ 1% dried Neem leaf powder, T_M = Control+ 1% dried Moringa leaf powder, T_C = Control + 1% Citric acid

^{abc} means bearing dissimilar superscript in same row differ significantly at the level of 5%

Blood profile

Creatinine level was significantly high in control group of birds (Table 4) and lower level was observed in T_M. Total protein (4.40 g/dl) and globulin (2.91 g/dl) were high ($p < 0.05$) in T_M where albumin (2.48 g/dl) were higher in T_N. Calcium and phosphorus level were Cholesterol and glucose level were significantly affected by the inclusion of dried leaf powder and citric acid in the diet. Total cholesterol, LDL as well as LDL/HDL ratio were lower ($p < 0.05$) in T_M. Moreover,

weight gain of broiler and also mentioned that supplementation of citric acid at a level of 3 % can enhance the feed conversion ratio. Moreover, citric acid is an organic acid which create a suitable gut environment for growth enhancing bacteria by reducing the pathogenic bacteria that improve the feed utilization efficiency of broiler (Baghban-Kanani *et al.,* 2019; Hasan *et al.,* 2016). But FCR, ME intake per gram of gain, CP intake per gram of gain were lower and energy efficiency ratio was lower in T_M. Osama

(2020) also found better body weight gain supplementing Moringa seed powder that supports the results of the present experiment. Another researcher, Banjo (2012) conducted an experiment supplementing different levels of moringa leaf (1%, 2% and 3%) and found better body weight gain at 2% moringa leaf supplementation. Inclusion of extruded hemp in broiler feed increases the growth performance by enhancing the availability of certain enzymes for proper nutrient utilization (khan, 2010). Medicinal plant has antioxidant properties, some important amino acids which enhances the production of certain enzymes that increase energy and protein efficiency ratio in broiler. Alpha linoleic acid is high in moringa (Moyo, 2011) and for enhancement in carcass yield, ascorbic and tocopherol of moringa may be responsible (Hekmat *et al.,* 2015; Khan *et al.,* 2012).

capacity in $T_{M.}$ Moreover, decreasing trend in p^H of broiler meat after 24 hours of storage is very high in control and T_C and lower in neem and moringa supplemented group of birds. Faster reduction in p^H makes the meat dry and pale may be due to the reduction of water holding capacity of meat that`s why water holding capacity is high and drip loss and cooking loss is low in T_N and $T_{M.}$ Micronutrient, essential oil and antioxidant of neem increase the utilization of feed protein which ultimately enhances the protein content of broiler meat in $T_{N.}$

Medicinal herb supplementation affects the blood metabolites of broiler and many herbs can reduce the total cholesterol, LDL and trigger HDL production. According to khan *et al.,* 2012, herb can alter the cholesterol production process in liver and convert the cholesterol into bile acid through limiting

Table 4: Blood Metabolites

Parameter	T_0	T_N	T_M	T_C	*p*-value
Creatinine (mg/dl)	0.28 ±0.02	0.23±0.003	0.18±0.05	0.20±0.03	0.076
Total protein (g/dl)	3.90^{c}±0.12	4.20^{b}±0.21	4.40^{a}±0.19	4.28^{b}±0.25	0.001
Albumin (g/dl)	1.59^{bc}±0.23	2.48^{a}±0.15	2.08^{b}±0.19	1.24^{c}±0.14	0.004
Globulin (g/dl)	2.17^{b}±0.32	2.12^{b}±0.12	2.91^{a}±0.12	1.67^{c}±1.09	0.036
Phosphorus (mg/dl)	7.06^{c}±0.71	8.20^{b}±1.11	9.38^{a}±1.22	8.24^{b}±0.89	0.025
Calcium (mg/dl)	4.27^{b}±1.10	4.35^{b} ±2.2	4.76^{a} ±1.6	4.15^{c}±1.10	0.033
Urea (mg/dl)	8.04±0.69	6.19±0.59	5.32±0.62	6.05±0.23	0.092
Uric acid (mg/dl)	4.75±0.71	4.28±0.94	4.25±0.61	4.36±0.32	0.065
Total Cholesterol (mg/dl)	102.9^{a} ±9.12	92.85^{b} ±8.35	76.18^{c}±2.73	85.9^{bc} ±6.57	0.002
HDL (mg/dl)	90.23±5.53	82.75±6.21	95.61±3.98	86.66±6.11	0.068
LDL (mg/dl)	79.34^{a}±4.76	65.67^{b}±3.55	35.55^{c}±4.11	45.48^{bc}±3.05	0.002
LDL/HDL	0.88^{a}±0.22	0.80^{a}±0.17	0.37^{c}±0.05	0.52^{b}±0.08	0.001
Glucose (mg/dl)	170.43^{a}±0.58	150.65^{b}±0.33	135.71^{bc}±0.21	140.51^{c}±0.71	0.041

T_0 = Control group, T_N = Control+ 1% dried Neem leaf powder, T_M = Control+ 1% dried Moringa leaf powder, T_C = Control + 1% Citric acid

^{abc} means bearing dissimilar superscript in same row differ significantly at the level of 5%

Carcass Quality and Blood Metabolites

Meat p^H is correlated to meat color and this is an important factor for consumer preference (Kostadinovic *et al.,* 2015). Low meat p^H is responsible for acidic meat as well as dark, firm and dry meat results from higher p^H of meat (Laudadio *et al.,* 2011). Moreover, according to Tashla (2019) normal p^H for broiler meat is 5.6 to 6.1 and in the present experiment, p^H is within the range but have no significant effects of medicinal herb and organic acid supplementation on meat p^H. In the present experiment, p^H level is not significantly affected by the supplementation of medicinal herb and citric acid but numerically lower in $T_{M,}$ but water holding capacity is high in both thigh and breast meat than control. The reason behind that may be the p^H value of meat was within the range. Puvaca (2011) stated that, lower p^H which means acidic meat have lower water holding capacity which supports the result of the present experiment. Young (2003) also reported that antioxidant enhance water holding capacity of meat and in this experiment, antioxidant present in moringa leaf may be responsible for higher water holding

the action of HMG-CoA reductase and fatty acid synthase. Furthermore, Balami (2018) also found low total cholesterol, triglycerides and LDL in broiler blood serum where moringa is supplemented which is similar to our findings. High concentration of polyphenol, flavonoid, phenolic compound in Moringa shows hypercholesterolaemic effects (Verma *et al.,* 2009) and high fiber of moringa also limit absorption of triglycerides and cholesterol from intestinal tracts (Mandal *et al.,* 2014). Flavonoid and alkaloids of hemp also helps in lowering the LDL cholesterol (Ramadan *et al.,* 2007). Moringa can prevent the catabolism of protein by limiting the secretion corticosterone which ultimately enhances protein level in blood (Luqman *et al.,* 2012). The increase in serum protein level is the reflection of maximum metabolism of feed protein (Sirvydis *et al.,* 2006) and Teye (2013) was found more serum protein level in broiler than control group when supplementing moringa leaf. The relation between serum urea and protein concentration is vice versa. When the concentration of serum protein is high, urea level becomes low and the reason behind that is the efficient absorption and utilization of dietary protein

that may due to the presence of micronutrient in moringa (Hussein *et al.,* 2019). Moringa is a rich source of mineral and protein and for this reason, calcium and phosphorus level in blood serum is higher in $T_{M.}$

Conclusion

The most important challenge in present broiler industry is to produce safe broiler, fulfilment of the consumer preference along with the reduction of production cost as well as cope up the demand of broiler meat. In this situation, inclusion of phytobiotics in broiler ration is very impressive. Phytobiotics like neem and moringa leaf can be used in broiler feed for the safe and more nutritious broiler meat.

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POULTRY STUDIES

RESEARCH PAPER

The Impact of Strain and Cage Type on the Welfare of Laying Hens in Different Seasons*

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Abstract

The aim of this study was to determine the effects of strain and cage type on the welfare of laying hens in commercial flocks over different seasons. A 2 x 2 x 3 factorial design was used to evaluate the effects of strain (white and brown layers) and cage type (conventional and enrichable battery cages) on the welfare of laying hens over three seasons (winter, spring and summer). The Welfare Quality® Assessment Protocol for Poultry was used to assess the welfare of laying hens. The strain and cage type significantly affected the welfare of the laying hens, which varied according to the season. Hens from the brown strain exhibited higher occurrences of FPD, keel bone abnormalities, and feather loss on the head and neck. White strains displayed a greater percentage of hens with abnormalities in the toe, comb, and beak, along with pecking wounds on the comb and extensive feather loss on the back, rump, and belly. A higher prevalence of comb abnormalities was observed in conventional cages. Hens in enrichable cages had higher rates of FPD, toe, comb and beak abnormalities, as well as pecking wounds on the comb and extensive feather loss. As a result, it was concluded that enrichable cages have a more adverse impact on the welfare of laying hens, with welfare losses in enrichable cages being more pronounced in brown hens compared to white hens and with interactions between strain and cage type varying seasonally.

Introduction

Research on the effects of enriched or cage-free systems on laying hen performance, health and welfare is ongoing. (Welfare Quality, 2009; Riber and Hinrichsen, 2016; Grafl et al., 2017). Over the past quarter of a century, there has been an increase in public and consumer interest in the welfare of laying hens (Hester, 2014). Because of their demands for high welfare standards for chickens, Directive 1999/74/EC on the protection of laying hens in the European Union came into force and was transposed into national law in Türkiye (Official Journal 29183 of 22 November 2014; as amended by Official Journal 31987 of 18 October 2022). Conventional cages, which severely restrict hens' freedom of movement, are banned. It also encourages the development of laying hen systems with higher welfare standards (Dawkins 2003).

Efforts to develop an industrial model for cage-free systems with outdoor access are still ongoing, and cage-free systems provide hens with the highest degree of freedom to move (Heerkens et al., 2015). However, challenges associated with egg production, cost efficiency, egg quality and animal health are described for these systems (Hartcher and Jones, 2017).On the other hand, even though enriched cages do not offer the same degree of freedom of movement to the hens, they have become a preferred housing system compared to conventional cages. This preference stems from increased comfort through features like claw trimming, nesting, perching and increased cage space (Hartcher and Jones, 2017;

Abrahamsson and Tauson, 1997). However, with the ban on conventional cages (the final deadline for which is 1 January 2026 in Turkey), the egg industry is facing a major cage system conversion, which will require significant economic resources. Enriched cages are gradually being purchased by poultry farms that are unable to convert their entire capacity at once. Enriched cages can be converted to enrichable cages by removing equipment such as perches, nests and claw-shortening devices, which can be integrated in a modular manner (Heflin et al., 2018; Alig et al., 2023). There is a limited amount of research on the effects of cage systems on the welfare of laying hens. In particular, there is a need for research to investigate the effects of enrichable cages on the welfare of laying hens. In addition, there is little research on how laying hen welfare is affected by cage type, strain and season, or the interactions between these factors.

Materials and Methods

Animals and Experiment Design

The study was conducted in four egg-producing poultry farms with two housing systems for laying hens in Afyonkarahisar. A 2 x 2 x 3 factorial design was used to investigate the effects of strain (white and brown laying hens) and cage type (conventional and enrichable battery cages) on the welfare of commercial laying hens during three seasons (winter, spring and summer). Super Nick and Nick Brown hens were housed in conventional cages (5 rows/5 or 6 tiers, and 8 or 9 birds per cage) and Hy-Line and Nick Brown hens were housed in enrichable cages (6 rows/6 or 7 tiers, and 18 or 19 birds per cage). Standard layer diets were fed to white (16-17% protein, 2600-2840 Kcal metabolic energy) and brown (15.8% protein, 2600 Kcal metabolic energy) strain laying hens. Animal care, indoor climate, air quality and lighting (16.5 L / 7.5 D) were controlled by automated systems. The Hy-Line birds were beak-trimmed with a hot blade at 9 days of age on the farm and the birds from the other 3 stains were beak-trimmed with infrared at 1 day of old in the hatchery. The birds were cared for and managed according to the breeder's guidelines (Hy-Line W-80 2016; Brown Nick 2016; Super Nick 2017). All layers had received a routine field vaccination programme. The study was approved by the Institutional Animal Ethics Committee of Afyon Kocatepe University (20 June 2017, No. AKUHADYEK-244-17). The results of this study on hen performance and mortality will be published in another paper (Kaba and Bozkurt, 2023).

Animal Welfare Assessment

The timing of the welfare assessments was planned according to the animal health and biosecurity policies and the production and marketing schedules of all the farms, so that the welfare assessments of the laying hens in the commercial flocks of the four farms could be carried out simultaneously. The welfare of laying hens in all flocks was assessed three times when they were between 36 and 56 weeks old. The welfare assessment was carried out on a total 969 laying hens in the winter season (December) the spring season (March) and the summer season (June) respectively. The sampling method and sample size per season measurement were based on the Welfare Quality (2009) standards and other on-farm welfare assessment methods to ensure reliable results for the welfare assessment of all flocks (Rodenburg et al., 2008; Casey-Trott et al., 2017). From each enrichable cage system, 5 enrichable cages were sampled. To obtain a representative average with a sample size comparable to the number of enrichable cages, 10 conventional cages were sampled from each of two other layer flocks kept in conventional cage systems. Thus, 30 cages were sampled in each season. Cages were randomly sampled from different rows (near the wall or in the centre of the poultry house) and from each level (top to bottom tiers) to ensure uniform sampling of different cage positions and within-cage conditions (Widowski et al., 2017). All birds in the sampled cages were scored. All hens in the sampled cages were carefully removed without frightening or injuring them and each was inspected and scored for head, foot and breast abnormalities and body feather damage. When the welfare assessment was repeated each season, samples were taken from other cages that had not been assessed in the previous season.

The method used to assess the welfare of laying hens was based on the welfare principles and criteria of The Welfare Quality® Assessment Protocol for Poultry (Welfare Quality, 2009). In addition, previous research on laying hen welfare was also considered (Heerkens et al., 2016; Grafl et al., 2017; Widowski et al., 2017). For the principle of good feeding, a resource-based measure was used; the feeder space per hen (cm/hen) was calculated by dividing the length of the feeders by the total number of hens in each cage (linear feeders extending in front of the cages). For good housing, the space allowance per hen (cm²/hen) was determined by dividing the total cage area by the total number of hens in the cage.

Each hen was scored for the presence of foot pad dermatitis (FPD), keel bone abnormalities, eye pathology, toe damage, comb abnormalities and beak trimming and beak abnormalities as measures of good health. The condition of the foot pads for FPD was scored for the absence of injury and disease (score 0: no lesions; score 1: mild swelling, necrosis or chronic bumblefoot with no pain and small superficial wounds ≤0.5 cm in diameter. The hens' toes (score 0: no signs of toe damage, score 1: toe damage, deformity or malformation), and eyes (score 0: no signs of eye pathology, score 1: swelling, lesions on the skin around

the eye, eye discharge, and closed eye) were examined and the pathology and abnormalities observed were scored. The breast region of the hens was carefully observed and fingers were run along the side and over the keel bone to examine it. The condition of the keel bone was then scored (score 0: no deformities or fractures, score 1: deviation, fracture, collapse, deformities or thickened areas present on the sternum or keel bone). The beaks of the hens were examined and abnormalities associated with beak trimming were scored (score 0: no abnormalities, score 1: beak not trimmed or with mild to moderate abnormalities, score 2: severe trimming, obvious abnormalities) as a measure of the absence of pain caused by management procedures. Abnormalities of the hens' comb were scored (score 0: no abnormalities, score 1: slightly pale colour or slight discolouration on comb, score 2: bruising or large areas of different colour on comb). Signs potentially associated with aggressive pecking on the comb of the hens (as a welfare criterion and expression of social behaviors) were scored (score 0: no evidence of pecking wounds, score 1: few pecking wounds or scars less than 3, score 2: numerous wounds, new or healing wounds more than 3). As a measure of the same welfare criteria, the hens were assessed and scored separately for feather loss and feather damage in three body parts: head-neck, back-rump and belly and around the cloaca (score 0: complete feather cover and no feather loss, score 1: moderate feather damage or loss, at least one bare skin area <5 cm in diameter, score 2: excessive feather damage or loss, at least one bare skin area ≥5 cm in diameter).

Statistical analysis

Two-way analysis of variance (ANOVA) was used to analyse the data collected in terms of feeder space and cage area per bird for each season. The chi-squared test was used to evaluate the data related to the occurrence of footpad dermatitis (FPD), keel bone abnormalities, eye pathology, toe damage, comb abnormalities, beak trimming and beak abnormalities, comb pecking wounds and feather damage in each season. Data analysis was performed using the SPSS version 21.0 for Windows. Differences were considered statistically significant when the significance level was less than 0.05.

RESULTS

The results for feeder space and space allowance are shown in Table 1. The effect of stain on feeder space was found to be significant in the spring season and overall ($P<0.05$, $P<0.001$); however, the effect of cage type was not significant in any of the seasons. The feeder space was smaller for white-strain hens, and a significant interaction between strain and cage type was observed for the feeder space. This interaction was particularly notable during the summer season ($P<0.01$). Strain and cage type considerably impacted the space allowance in the cages ($P<0.001$). Space allowance was influenced by strain in the spring and in overall ($P < 0.05$, $P < 0.001$). The effect of cage type on space allowance was insignificant in seasonal groups but it was significant in overall ($P<0.001$). Among the different strain flocks, the allocated living area per hen was greater for brown-layer hens. Notably, during the spring and summer, the cage area provided for brown hens was significantly larger than white hens ($P < 0.01$), with a difference of 74 cm² favoring brown hens. Enrichable cages also provided more space per bird than conventional cages, with an average of 39-52 cm² more cage area in enrichable cages. The interaction between strain and cage type was particularly notable during the summer, with similar space allowances in both cage types for brown hens (474.20 and 474.05 cm²). In contrast, the space allowances for white hens housed in conventional and enrichable cages were 377.03 and 461.66 cm², respectively. White and brown laying hens housed in enrichable cages had similar feeder space (7.74 and 8.01 cm), whereas, in conventional cages, there was a significant difference between these values between two strains of layers (6.61 and 8.32 cm).

The results related to the effects of strain and cage type on FPD, toe damage, keel bone abnormalities and eye pathologies in different seasons are given in Table 2. The rate of hens with PFD was affected by strain in summer and cage type in winter and spring. Toe damage was significantly influenced by strain in winter and spring ($P<0.01$) and by cage type in spring ($P<0.05$). Regardless of season, both FPD and toe damage were generally affected by strain and cage type ($P<0.05$, $P<0.01$, $P<0.001$). The effect of strain and cage type on eye pathologies was not significant in any season. Keel bone abnormality was affected by strain in spring and summer ($P< 0.01$), whereas cage type had no significant effect.

The results concerning the effects of strain and cage type on comb and beak abnormalities and comb peck wounds in different seasons are given in Table 3. Comb abnormalities were significantly influenced by strain in winter ($P<0.001$) and by cage type in spring ($P<0.05$). However, regardless of the seasonal effect, strain and cage type influenced comb abnormality ($P<0.05$, $P<0.01$). Strain significantly affected beak abnormality in spring and summer ($P<0.001$), while cage type didn't show any significant effects during across the seasons. Disregarding the seasonal effect, beak abnormality was only significantly ($P<0.001$)

influenced by strain. Strain significantly influenced comb pecking wounds in all three seasons (P<0.001, P<0.05). Although the within-season effects are insignificant, the overall assessment of all seasons showed that strain and cage type influenced comb pecking wounds (P<0.05).

Table 4 shows the results of the effects of strain and cage type on feather damage and feather loss on three individual body parts in different seasons. Head and neck feather damage was significantly affected by strain in all seasons (P<0.05, P<0.01) and by cage type in spring and summer (P<0.05, P<0.001). The effects of strain (P<0.001) and cage type (P<0.01, P<0.001) on back-rump feather damage were significant in winter and summer. Belly feather damage was strongly influenced by strain in winter and summer (P<0.05), and the effects of cage type were significant only in summer (P<0.05). In the overall assessment, regardless of season, plumage damage was significantly (P<0.001) influenced by strain for back-rump and head-neck, and by cage type for back-neck and belly (around the cloaca) (P<0.001, P<0.01).

DISCUSSION

In this study, feeder space per bird was significantly influenced by strain and the interactions between strain and cage type. White and brown laying hens housed in enrichable cages had similar feeder space (7.74 and 8.01 cm), whereas, in conventional cages, there was a significant difference between these values (6.61 and 8.32 cm). Feeder space in all experimental groups was therefore less than required by EU legislation (10 cm per hen), and was lowest for the conventionally housed white hens in particular (Council Directive 1999/74/EC) (Council Directive, 1999). In terms of the principle of good feeding, insufficient feeder space for all birds can lead to detrimental outcomes due to increased competition between hens for access to feed (Thogerson et al., 2009). The legal cage area requirement (750 cm^2 per hen) was not met by both white and brown hens in conventional and enrichable cages. Brown hens in enrichable cages (499.77 cm^2/hen) were found to have less space than white hens (514.33 cm^2), especially in summer. These results showed that cage overcrowding increased for the larger brown hens in the enrichable cages. Mortality was already higher in enrichable cages, and the cumulative weekly mortality rate of white and brown breeds housed in enrichable cages was 0.34% and 0.36%, respectively (Kaba and Bozkurt, 2023).

Hens of the White strain had a higher prevalence of abnormalities in the toe, beak and comb than those of the Brown strain. Toe and comb abnormalities were more pronounced in winter, while the prevalence of beak abnormalities was higher in spring and summer. The hot blade beak trimming method and applicator errors in the Hy-Line birds may be responsible for the higher incidence of beak abnormalities in the White strain hens, as the beaks of all the other hens were trimmed by infrared trimming in the hatchery. More consistent beak lengths and fewer abnormalities, such as cracks, asymmetric regrowth and blisters were reported in birds whose beaks were trimmed using infrared compared to birds whose beaks were trimmed using a hot blade (Carruthers et al., 2012; Glatz and Underwood, 2020). The prevalence of comb peck wounds was highest in white strain hens across all seasons, suggesting a higher incidence of aggressive pecking in white hens. White strain hens with a higher prevalence of toe abnormalities are thought to be more reactive to stressors and experience more panic, resulting in damage to their toes and claws as they become entangled in the grids on the cage floor (Fraisse and Cockrem, 2006; Janczak and Riber, 2015). The absence of wounds is an important welfare criterion (Grafl et al., 2017), as it is essential for the health and welfare of laying hens. Some studies have suggested that anxiety levels may vary between strains and that the acquisition of anxiety may be reduced or enhanced by the experience of birds in commercial conditions (Hocking et al., 2001).

Foot pad dermatitis (FPD) was common in all hens tested (no birds were scored 3). However, it was particularly high in the brown flocks. Overall, the proportion of brown layer hens with lesions on the foot pads was 34.2 % and this rate increased to 54.4 % during the summer months. The significant effect of strain on FPD was also reported by Niebuhr et al. (2009). It may also have been influenced by the fact that the brown hens had a heavier body weight than the white hens. In this study, the smaller amount of space available per brown hen in the enrichable cages may also have contributed to this condition (Niebuhr et al., 2009). FPD lesions can appear as hyperkeratosis and dermatitis on the foot pads, usually due to prolonged ground contact by the birds. These painful lesions, especially in the case of advanced lesions, are detrimental to the health and welfare of the birds (Abrahamsson and Tauson, 1997; Riber and Hinrichsen et al., 2016; Rørvang et al., 2019; Oliveira et al., 2019). In conventional cages, the percentage of laying hens with FPD lesions was significantly higher only in winter (17.1%) than in enrichable cages. Similarly, Grafl et al (2017) reported poorer feather condition and increased skin and footpad lesions in hens during the winter months. The lower rate of footpad lesions in conventional cages may be due to the restrained behavior of the hens due to the limited cage space (Hartcher and Jones, 2017). In spring and summer, the rate of hens with FPD is higher in enrichable cages (24.6 and 2.9 % higher). Particularly in spring, the prevalence of FPD lesions (61.5%), toes (34.8%), comb abnormalities (46%) and comb peck

Table 1. Effect of strain and cage type on feeder space and space allowance per hen in the cages in different seasons

		Winter			Spring			Summer			General	
			Feeder space (cm/hen)	Space allowance (cm^2/hen)		Feeder space (cm/hen)	Space allowance (cm^2/hen)		Feeder space (cm/hen)	Space allowance (cm^2/hen)		Feeder space (cm/hen)	Space allowance (cm^2/hen)
Stain	Cage type	n	Mean	Mean	n	Mean	Mean	n	Mean	Mean	n	Mean	Mean
White		15	6.74	391.32	15	6.94	402.89	15	7.25	421.51	45	6.97	405.24
Brown		15	7.75	449.59	15	8.24	477.43	15	8.55	495.43	45	8.18	474.15
	Conventional	20	7.15	407.48	20	7.52	428.17	20	7.74	441.19	60	7.47	425.61
	Enrichable	10	7.44	446.41	10	7.74	464.14	10	8.22	493.02	30	7.80	467.86
SEM			0.234	13.739		0.207	12.291		0.228	13.323		0.129	7.599
R^2			0.181	0.216		0.361	0.373		0.418	0.441		0.286	0.310
P value													
Strain			0.053^{-}	0.055^{-}		0.018*	0.021*		0.068^{-}	0.079^{-}		0.000***	0.001***
Cage			0.539^{-}	0.168^{-}		0.594^{-}	0.155^{-}		0.305^{-}	0.063^{-}		0.204^{-}	0.007**
Strain x Cage			0.704^{-}	0.743^{-}		0.081^{-}	0.096^{-}		0.008**	0.009**		0.005**	0.007**

*:P<0.05, **:P<0.01, ***:P<0.001 -: Non significant

Table 2. Effect of strain and cage type on FPD, toe damage and, keel bone abnormalities and eye pathologies in different seasons

				Winter		Spring		Summer		General	
Measures	Strain	Cage type	χ^2	Score 0	Score 1	Score 0	Score 1	Score 0	Score 1	Score 0	Score 1
FPD	White			86.5	13.5	55.9	44.1	82.3	17.7	75.2	24.8
	Brown			87.0	13.0	45.6	54.4	65.4	34.6	65.8	34.2
	General			86.8	13.2	50.8	49.2	74.0	26.0	70.6	29.4
			P	0.902^{-}		0.066^{-}		0.001***		0.001***	
		Conventional		82.9	17.1	63.1	36.9	75.5	24.5	73.8	26.2
		Enrichable		90.4	9.6	38.5	61.5	72.6	27.4	67.5	32.5
		General		86.8	13.2	50.8	49.2	74.0	26.0	70.6	29.4
			P	0.046*		0.000***		0.551^{-}		0.031*	
	White			84.2	15.8	63.4	36.6	75.6	24.4	74.6	25.4
Toe	Brown			94.2	5.8	78.8	21.2	82.4	17.6	85.0	15.0
	General			88.9	11.1	71.0	29.0	78.9	21.1	79.7	20.3
			P	0.004**		0.002**		0.135^{-}		0.000***	
		Conventional		91.8	8.2	76.9	23.1	81.8	18.2	83.4	16.6
		Enrichable		86.2	13.8	65.2	34.8	76.2	23.8	76.0	24.0
		General		88.9	11.1	71.0	29.0	78.9	21.1	79.7	20.3
			P	0.111^{-}		0.021*		0.222^{-}		0.004**	
Eye	White			97.7	2.3	96.9	3.1	98.2	1.8	97.6	2.4
	Brown			99.4	0.6	92.5	7.5	97.5	2.5	96.4	3.6
	General			98.5	1.5	94.7	5.3	97.8	2.2	97.0	3.0
			P	0.216^{-}		0.079^{-}		0.672^{-}		0.283^{-}	
		Conventional		97.5	2.5	93.1	6.9	97.5	2.5	96.0	4.0
		Enrichable		99.4	0.6	96.3	3.7	98.2	1.8	98.0	2.0
		Cage type total		98.5	1.5	94.7	5.3	97.8	2.2	97.0	3.0
			P	0.157^{-}		0.208^{-}		0.672^{-}		0.075^{-}	
Keel bone	White			96.5	3.5	96.3	3.7	99.4	0.6	97.4	2.6
	Brown			98.7	1.3	88.1	11.9	93.7	6.3	93.4	6.6
	General			97.5	2.5	92.2	7.8	96.6	3.4	95.5	4.5
			P	0.199^{-}		0.006**		0.005**		0.003**	
		Conventional		96.8	3.2	90.6	9.4	95.6	4.4	94.3	5.7
		Enrichable		98.2	1.8	93.8	6.2	97.6	2.4	96.5	3.5
		General		97.5	2.5	92.2	7.8	96.6	3.4	95.5	4.5
			P	0.426^{-}		0.290^{-}		0.331^{-}		0.099^{-}	

*:P<0.05, **:P<0.01, ***:P<0.001, -: Non significant, **FPD**: Food pad dermatitis

Table 3. Effect of strain and cage type on comb and beak abnormalities and comb pecking wounds in different seasons.

				Winter			Spring			Summer			General		
Measures	Strain	Cage type	χ^2	Score 0	Score 1	Score 2	Score 0	Score 1	Score 2	Score 0	Score 1	Score 2	Score 0	Score 1	Score 2
	White			67.8	25.2	7.0	52.8	42.9	4.3	60.4	28.6	11.0	60.5	32.0	7.5
Comb	Brown			85.1	13.0	1.9	56.9	35.0	8.1	65.5	27.0	7.5	68.9	25.2	5.9
	General			76.0	19.4	4.6	54.8	38.9	6.3	62.8	27.9	9.3	64.6	28.7	6.7
			P		0.001***			0.187-			0.491-			0.023*	
		Conventional		79.1	15.8	5.1	59.4	31.8	8.8	67.3	23.9	8.8	68.6	23.9	7.5
		Enrichable		73.0	22.8	4.2	50.3	46.0	3.7	58.5	31.7	9.8	60.8	33.3	5.9
		General		76.0	19.4	4.6	54.8	38.9	6.3	62.8	27.9	9.3	64.6	28.7	6.7
			P		0.281-			0.014*			0.243-			0.005**	
Beak	White			28.7	39.8	31.5	17.4	46.6	36.0	25.6	36.0	38.4	24.0	40.7	35.3
	Brown			35.1	31.2	33.8	53.1	33.1	13.8	49.7	37.1	13.2	46.1	33.8	20.1
	General			31.7	35.7	32.6	35.2	39.9	24.9	37.5	36.5	26.0	34.8	37.4	27.8
			P		0.241-			0.000***			0.000***			0.000***	
		Conventional		34.8	34.2	31.0	38.8	35.6	25.6	37.7	35.2	27.0	37.1	35.0	27.9
		Enrichable		28.7	37.1	34.1	31.7	44.1	24.2	37.2	37.8	25.0	32.5	39.6	27.8
		General		31.7	35.7	32.6	35.2	39.9	24.9	37.5	36.5	26.0	34.8	37.4	27.9
			P		0.501-			0.266-			0.868-			0.240-	
Comb	White			38.6	49.1	12.3	62.7	34.8	2.5	53.0	27.5	19.5	51.2	37.3	11.5
pecking	Brown			70.8	26.6	2.6	48.1	44.4	7.5	49.7	39.6	10.7	56.0	37.0	7.0
wounds	General			53.8	38.5	7.7	55.5	39.5	5.0	51.4	33.4	15.2	53.6	37.2	9.2
			P		0.000***			0.011*			0.019*			0.041*	
		Conventional		58.9	33.5	7.6	61.9	33.1	5.0	54.1	32.7	13.2	58.3	33.1	8.6
		Enrichable		49.1	43.1	7.8	49.0	46.0	5.0	48.8	34.1	17.1	49.0	41.0	10.0
		General		53.8	38.5	7.7	55.5	39.5	5.0	51.4	33.4	15.2	53.5	37.2	9.3
			P		0.185-			0.057-			0.525-			0.014*	

*:P<0.05, **:P<0.01, ***:P<0.001, -: Non significant

Table 4. Effect of strain and cage type on plumage damage on three individual body parts in different seasons

				Winter			Spring			Summer			General		
Measures	Strain	Cage type	χ^2	Score 0	Score 1	Score 2	Score 0	Score 1	Score 2	Score 0	Score 1	Score 2	Score 0	Score 1	Score 2
Head-neck	White			88.9	11.1	0.0	58.4	26.1	15.5	54.3	29.9	15.9	67.5	22.2	10.3
	Brown			79.2	19.5	1.3	45.0	38.1	16.9	36.5	47.2	16.4	53.3	35.1	11.6
	General			84.3	15.1	0.6	51.7	32.1	16.2	45.5	38.4	16.1	60.6	28.5	10.9
			P	0.032*				0.039*			0.003**			0.000***	
		Conventional		82.9	17.1	0.0	58.8	30.0	11.3	43.4	31.4	25.2	61.6	26.2	12.2
		Enrichable		85.6	13.2	1.2	44.7	34.2	21.1	47.6	45.1	7.3	59.6	30.7	9.8
		General		84.3	15.1	0.6	51.7	32.1	16.2	45.5	38.4	16.1	60.6	28.5	10.9
			P		0.248-			0.016*			0.000***			0.206-	
Back-rump	White			96.5	3.5	0.0	59.0	24.8	16.1	39.6	33.5	26.8	65.5	20.4	14.1
	Brown			81.8	17.5	0.6	50.6	34.4	15.0	48.4	46.5	5.0	60.0	33.0	7.0
	General			89.5	10.2	0.3	54.8	29.6	15.6	44.0	39.9	16.1	62.8	26.5	10.6
			P		0.000***			0.169-			0.000***			0.000***	
		Conventional		96.5	4.4	0.0	56.9	25.0	18.1	31.4	42.1	26.4	61.2	23.9	14.9
		Enrichable		83.8	15.6	0.6	52.8	34.2	13.0	56.1	37.8	6.1	64.4	29.1	6.5
		General		89.5	10.2	0.3	54.8	29.6	15.6	44.0	39.9	16.1	62.8	26.5	10.6
			P		0.002**			0.146-			0.000***			0.000***	
Belly	White			98.8	1.2	0.0	64.6	26.1	9.3	65.2	25.6	9.1	76.6	17.3	6.0
	Brown			92.9	6.5	0.6	68.8	24.4	6.9	76.7	22.0	1.3	79.3	17.8	3.0
	General			96.0	3.7	0.3	66.7	25.2	8.1	70.9	23.8	5.3	77.9	17.5	4.5
			P		0.022*			0.640-			0.003**			0.070-	
		Conventional		98.1	1.9	0.0	71.9	19.4	8.8	76.7	20.8	2.5	82.2	14.0	3.8
		Enrichable		94.0	5.4	0.6	61.5	31.1	7.5	65.2	26.8	7.9	73.8	20.9	5.3
		General		96.0	3.7	0.3	66.7	25.2	8.1	70.9	23.8	5.3	77.9	17.5	4.5
			P		0.152-			0.055-			0.027*			0.007**	

*:P<0.05, **:P<0.01, ***:P<0.001, -: Non significant

wounds (41%) were higher in the hens housed in enrichable cages. In addition, the prevalence of keel bone and eye abnormalities was slightly higher in these hens in all seasons, but the differences between cage types were not statistically significant. These results were consistent with the report of Niebuhr et al. (2009), who reported a positive correlation between stocking density and FPD but these results contrasted were contrasted with the findings of Hester (2014).

These results are consistent with the report of Niebuhr et al. (2009), who reported a positive correlation between stocking density and FPD, but contrast with the findings of Hester (2014). The results of this study regarding foot and toe problems may be related to the lack of access to perches in enrichable cages, as perches contribute to the health of foot pads, toes and claws (Riber and Hinrichsen, 2016). However, the proportion of hens with keel bone and eye abnormalities was lower in enrichable cages than in conventional cages. This finding contradicts Riber and Hinrichsen (2016), who reported a higher prevalence of keel bone fractures in enriched cages. Keel bone fractures and deformities significantly restrict the behavior of commercial laying hens and compromise their welfare (Stratmann et al., 2015; Riber and Hinrichsen, 2016). The high incidence of both FPD and foot pad deformities in Brown strain hens suggests a possible link between the development of these two traits. This argument is supported by Heerkens et al. (2015), who reported a positive correlation between sternal fracture prevalence of sternal fractures and foot pad lesions. Already, in spring, the proportion of brown strain hens with keel bone abnormalities (11.9% and 6.3%) and comb pecking wounds was higher in spring (44.4 and 7.5% for score 1 and 2) than in summer (39.6 and 10.7% for score 1 and).Hens housed in conventional cages had a higher prevalence of comb abnormalities (7.5 %), but fewer toe abnormalities and pecking comb lesions than those housed in enrichable cages. Traumatic damage and deformities to the keel bones can cause to acute or chronic pain and affect the welfare of the laying hens (Fleming et al., 2004; Nasr et al., 2012; Riber and Hinrichsen, 2016). Riber and Hinrichsen (2016) also reported that keel bone deformities in laying hens may have contributed to hens spending more time lying down and standing, resulting in increased footpad lesions.

The white strains showed a more moderate degree of feather loss in the head-neck area than the brown strains. However, the white strains were the most likely to show severe feather loss on the back rump. Particularly in the summer, 26.8% of white strain hens showed an excessive feather loss of feathers around the back rump and the belly (prevalence of moderate and severe feather loss were 25.6% and 9.1%, respectively). In conventional cages, white strain hens had the least space allowance per bird, which may explain the increased proportion of birds with severe feather loss. This is supported by Widowski et al. (2017), who reported that laying hens with lower space allowances tended to have poorer feather conditions. In addition, it has been suggested that these findings on feather loss may be related to stress responses and fear. A positive relationship between fear and pecking behavior has been reported (Rodenburg et al., 2004; Heerkens et al., 2016).In all seasonal periods, moderate and severe feather loss in the head-neck region was higher in brown strain hens, but the strain differences for these traits were more pronounced in the summer season (47.2% and 16.4% for scores 1 and 2). The percentage of brown strain hens with moderate and severe feather loss in the back-rump area was higher than that of the white strains in both winter (17.5% and 0.6%) and summer (46.5% and 5.1%). Feather damage around the cloaca area was less pronounced in brown hens and was only higher in winter (6.5% and 0.6%) compared to white strains. Overall, these feather damage findings could also be related to a genetic predisposition to severe feather pecking behaviour (Rodenburg et al., 2004), group size (Rørvang et al., 2019) or other stress-related risk factors (De Haas et al., 2013). For the hens in conventional cages, feather loss in the head-neck and belly and around the cloaca areas was not common; however, the percentage of hens with moderate and severe feather damage in the back-rump area was higher compared to those in enrichable cages (42.1% and 26.4% for Scores 1 and 2, respectively).

Compared to conventional cages, the percentage of hens with moderate or severe feather loss was higher enrichable cages in spring and summer for the head-neck area (34.2% and 45.1%, and 21.1% and 7.3%, respectively), in winter for the back-rump area (15.6 and 0.6%), and in summer for the belly (26.8 and 7.9%) in enrichable cages. Feather pecking behavior is abnormal in stressed hens (De Haas et al., 2013). This study suggests that enriched cages provide hens with more behavioural opportunities by increasing the amount of space available to the hens. However, it has been noted that enriched or enrichable cages might only partially accommodate the behavioral repertoire of the hens (Hartcher and Jones, 2017). These results suggest that the group size in enrichable cages is another important significant factor. Widowski et al. (2017) reported higher cumulative mortality rates in furnished cages that housing larger groups. The relationship between reduced feather condition and lower stocking densities has not been clearly established (Grafl et al., 2017; Widowski et al., 2017). However, the feather loss results suggest that brown strain hens in enrichable cages may experience greater stress, and their welfare may be lower than white strain hens

under the same conditions (Rodenburg et al., 2008). In general, it was determined that feather damage was higher in both conventional and enrichable cages. Feather pecking refers to hens pecking and pulling the feathers of others, posing a risk of cannibalism in the poultry industry, which threatens animal welfare, health, and production performance (Hartcher and Jones, 2017). However, the aging of the animals from winter to summer may have influenced the welfare characteristics studied, as the same experimental flocks were subjected to repeated welfare assessments during the winter, spring and summer seasons. Riber and Hinrichsen (2016) reported that the prevalence of sternal fractures increases with the age of laying hens. New research is needed to more clearly separate the factors of age and seasonality.

CONCLUSION

The strain and cage type significantly affected the welfare of the laying hens, which varied according to the season. Significant differences in scape allowance per hen in the cage, toe and beak abnormalities, keel bone problems comb peck wounds and feather damage were observed between white and brown strains for at least two seasons. Brown strain hens had more FPD, keel bone abnormalities and feather loss on the head and neck. The white strains had a higher percentage of hens with toe, comb and beak abnormalities, pecking wounds on the comb, and extensive feather loss on the back of the rump and belly. Hens in conventional cages had a higher incidence of comb abnormalities. Hens in enrichable cages had a higher proportion of hens with FPD, abnormalities in the toe, comb and beak and pecking wounds on the comb and extensive feather damage on the back rump and belly. In conclusion, enrichable cages had a more negative effect on the welfare of laying hens. The welfare losses in enrichable cages were more pronounced in brown hens than in white hens housed in the same cages, and the interactions between strain and cage type varied from season to season.

Conflict of interest: The authors declare that there are no actual, potential, or perceived conflicts of interest in this article.

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Author Contributions

First Author: Data Collection, Data Analysis and/or Interpretation, Literature Search, Writing Manuscript

Second Author: Conceptualization, Consultation, Data Analysis and/or Interpretation, Literature Search, Writing Manuscript, Critical Review

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