

## RESEARCH ARTICLE

# Comparison of Laying Performance, Egg Quality and Bone Characteristics of Commercial and Türk Laying Hen Genotypes Kept in a Free-Range System <sup>[1]</sup>

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[1] Presented at 5. International Food, Agriculture and Veterinary Sciences Congress as an oral presentation, 17-19 March 2023, Kars/Türkiye

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How to cite this article?

**Sözcü A, İpek A, Gündüz M:** Comparison of laying performance, egg quality and bone characteristics of commercial and Türk laying hen genotypes kept in a free-range system. *Kafkas Univ Vet Fak Derg*, 29 (5): 437-444, 2023. DOI: 10.9775/kvfd.2023.29284

Article ID: KVFD-2023-29284

Received: 28.02.2023

Accepted: 11.09.2023

Published Online: 12.09.2023

## ABSTRACT

This study was designed to compare of laying performance, egg quality and bone characteristics of commercial and Türk laying hen genotypes kept in free-range system. A total of 720 laying hens (Atabey, Lohmann White, Atak-S, Lohmann Brown; n=180 hens/genotype) were used in the experiment. Production performance was determined as the mean of egg production, egg weight, and FCR value between 54 and 66 weeks of age. Egg quality parameters and bone characteristics of tibia and femur were measured at 66 weeks of age. The mean value of egg production was found to be higher in Lohmann Brown and Lohmann White genotypes compared to Atak-S and Atabey genotypes between 54 and 66 weeks of age ( $P<0.01$ ). The brown eggs obtained from (Lohmann Brown, Atak-S) genotypes tended to be heavier than the white hen genotypes (Lohmann White, Atabey). The lowest mean value of FCR was observed in Lohmann White hens ranged from during the experimental period. The Lohmann Brown and Atak-S eggs obtained from ( $3.350 \text{ g/cm}^2$  and  $3.300 \text{ g/cm}^2$ ) had a stronger shell strength compared to the Lohmann White and Atabey ( $2.847 \text{ g/cm}^2$  and  $2.910 \text{ g/cm}^2$ ,  $P<0.01$ ). The breaking strength of tibia was found to be higher in brown hens ( $366.0 \text{ N}$  and  $381.2 \text{ N}$ ) than white hens ( $267.0 \text{ N}$  and  $322.2 \text{ N}$ ) ( $P<0.01$ ). These findings related to different genotypes could be instructive for arranging new management rules and nutritional advice for stronger eggshell and bone strength of hens in free range system.

**Keywords:** Eggshell breaking strength, Egg production, Laying hen, Tibia strength, Turkish genotypes

## INTRODUCTION

Egg production is one of the most significant component of animal production in Türkiye, approximately 19.7 billion eggs produced in a year from over 121 million laying hens in 2021 <sup>[1]</sup>. Egg production has been achieved mainly by three production systems including cage systems (conventional and enriched cage systems), free-range system and organic production system. Egg production comprises of 78.3% of all eggs produced from cage systems, 19.8% of all eggs from free-range systems and 1.9% of all eggs produced from organic systems during 2018 year in Türkiye <sup>[2]</sup>.

In recent years, consumers have increasingly sensitivity for food safety, animal welfare, sustainability and environmental protection in both European countries and Türkiye. These facts have caused new trends in animal production as like as other agricultural sectors. Therefore,

alternative egg production system (free-range and organic systems) have increasingly gained importance due to regarding animal rights and welfare issues <sup>[3]</sup>. Both of these alternative systems ensure free accessing to pasture that stimulates physical activities, benefit from natural light and sunshine and make possible to exhibit natural behaviors, for example perching, nesting, preening, foraging, dust bathing, and pecking <sup>[4]</sup>.

It is known that egg production and both exterior and interior egg quality are affected by many factors, such as breed, age, husbandry practices, feed composition, and nutritional content <sup>[5,7]</sup>. The most important quality criterias for the consumers are shell strength, albumen consistency and yolk color <sup>[8]</sup>. Furthermore, shell quality is largely depending on many factors including genetic factors, egg laying rate, age, health status, housing conditions, and nutrition <sup>[9,10]</sup>. The differences in nutritional factors among production system has a crucial role in the modulation of



bone mineral homeostasis, influencing the mineralization and mechanical strength of the bones and subsequently shell strength <sup>[11,12]</sup>.

The most important targets in egg production are to produce maximum eggs with saleable quality criterias and FCR, maintain a long-term laying persistence, and preventing skeletal disorders <sup>[13]</sup>. However, there are some statements that high-yielding hybrids kept in free-range or organic production systems with outdoor access could have some difficulties in adapting to the less controlled environmental conditions in outdoor areas and less equilibrated rations in free-range systems <sup>[14]</sup>. This could be resulted from these genotypes' suitability for production systems with environmental controlled conditions <sup>[15]</sup>. Therefore, it could be seen some losses in productivity, egg quality, health and welfare status of commercial layer hybrids kept in free-range system. To minimize these losses, it has been suggested preferring of native genotypes in free range systems, because of their robustness and rusticity <sup>[16]</sup> and higher adaptive capacity for varying geographical regions and local climate conditions in EU regulation 1804/99 <sup>[17]</sup> and the final recommendation of the Network for Animal Health and Welfare in Organic Agriculture <sup>[18]</sup>.

The aim of this study is to evaluate egg performance, egg quality and bone characteristics of commercial laying hen genotypes (Lohmann Brown and Lohmann White) and Türk laying hen genotypes (Atak-S and Atabey) in free range system. Atak-S (brown laying hens) and Atabey (white laying hens) are Türk local laying hens that have been developed by Ankara Poultry Research Institute in 2004 <sup>[19]</sup>. In this study, we focused on the significant differences for productivity, egg quality and bone characteristics of different genotypes during late laying period (54-66 weeks of age) in the free-range system.

## MATERIAL AND METHODS

### Ethical Approval

The care and use of animals were approved by the ethics committee of Bursa Uludag University and were in accordance with the laws and regulations of Türkiye (License Number 2019-05/09).

### Animals and Management Conditions

A total of 720 laying hens of two commercial laying hen genotypes (Lohmann Brown, Lohmann White) and two Türk laying hen genotypes (Atabey, white genotype; Atak-S, brown genotype) were used between 54 and 66 weeks of age. The experimental design included with three subgroups as pens which were considered as replicate for each genotype (n=3 pens/genotype, 60 hens/pen). At 54 weeks of age, the birds were individually weighed on a

digital scale with precision  $\pm 1$  g, and then were randomly allocated to the pens with 3 m  $\times$  7 m dimensions.

All birds were reared in a free-range system in accordance with the basic requirements of EU Directive 1999/74/EC <sup>[20]</sup>. According to these regulations, the free-range system had an indoor and outdoor pasture areas. Wood shavings material was used as litter material to cover pens' floor. Indoor area, all birds were provided as 6.5 cm feeder area per hen with circular plastic feeders and 5 cm drinker area per hen with plastic bell drinkers. Each pen was equipped with perches providing 18 cm perch length per hen, and nesting boxes (3.5 hens per nesting box). The outdoor area with a size of 350 m<sup>2</sup> per pen was covered by wire fences to keep out predators and a shelter. The stocking density was ensured as 2.86 hens per m<sup>2</sup> in indoor area and 5.83 hens per m<sup>2</sup> in outdoor area for each pen. The lighting regime in the pens was applied as 16 h lighting per 24 h period during experimental period.

A standard layer diet for free-range systems was used and the feed ingredients and nutrient composition of diet was analyzed according to <sup>[21]</sup> (Table 1). Feed and water were offered ad libitum throughout the experiment. The pasture area was comprised of 60% perennial ryegrass (*Lolium perenne*), 30% alfalfa (*Medicago sativa*), and 10% white clover (*Trifolium repens*). The birds could supplement their diets with pasturing and the living small creatures (insects, arthropods, etc.) in the foraging area.

**Table 1.** Composition and nutrient content of laying hen diet (54-66 weeks of age)

Feed Ingredients	%
Corn, grain	28.0
Wheat	32.9
Soybean meal, 48%	14.3
Sunflower meal, 32%	3.2
Milled alfalfa	8.6
Soybean oil	2.8
Sodium chloride	0.2
Limestone	8.2
Dicalcium phosphate	1.3
Premix*	0.5
Nutrient Composition	%
ME (kcal/kg)	2803
CP	16.6
Calcium	3.46
Phosphorus	0.48

\* 1 kg of premix includes the following components: Vit. A, 8.000 IU; Vit. D3, 2.000 IU; Vit. B2, 4 mg; Vit. B12, 10 mg; Vit. E, 15 mg; Vit. K3, 2 mg; Vit. B1, 3 mg; Niacin, 30 mg; Cal-D-pantothenic acid, 10 mg; Vit. B6, 5 mg; Folic acid, 1 mg; D-biotin, 0.05 mg; Vit. C, 50 mg; Choline chloride, 300 mg; Mn, 60 mg; Zn, 50 mg; Fe, 60 mg; Cu, 5 mg; Co, 0.5 mg; I, 2 mg; Se, 0.15 mg

## Data Collection

### Laying Performance

The pens were monitored daily basis for egg production (EP) until the end of the experiment. Egg production was calculated by dividing the number of eggs daily collected by the number of hens on the same day. Daily feed intake (DFI) and egg weight (EW) were recorded on a weekly basis. FCR was calculated on a weekly basis as the ratio between DFI and EP multiplied by EW. Egg mass (EM) was calculated as  $EM = (EP \times EW) / 100$ . The FCR was calculated as  $FCR = DFI / EM$ . The mean values for EP, EW, EM, DFI and FCR were given as 3 weeks interval (between 54-57, 58-61, and 62-66 weeks of age).

### Egg Characteristics

A total of 15 eggs from each genotype were randomly sampled to define external and internal egg quality parameters at 66 weeks of age. The measurements were performed 24 h after the eggs were laid. The eggs were weighed with  $\pm 0.01$  g precision (Model XB 4200C, Precisa Corp, Zurich, Switzerland), and then the length and width of the eggs were determined using a digital caliper with  $\pm 0.01$  mm precision (Mitutoyo, 300 mm, Neuss, Germany). The egg shape index was calculated with a formula of (egg width/egg length)  $\times 100$  [22]. Eggshell breaking strength ( $\text{kg}/\text{cm}^2$ ) was measured by using an eggshell force reader machine (Egg Force Reader, Orka Food Technology, Israel). The eggs were broken to separate the albumen and yolk, and the yolk weight was determined with  $\pm 0.01$  g precision.

The eggshells were carefully cleaned by washing process and then put in an oven at  $105^\circ\text{C}$  (Nüve FN-500, Ankara, Türkiye) during 24 h for drying process. Then, the eggshell weight was determined with  $\pm 0.01$  g precision. Albumen weight was calculated by subtracting yolk and shell weight from total egg weight. The ratio of yolk, albumen, and eggshell were given as a percentage of EW. Eggshell thickness was measured at three different points of the eggshell as specifically blunt, sharp end, and equator region, using a digital caliper with  $\pm 0.01$  mm precision. The eggshell thickness was given as the mean of measured three values.

The yolk diameter (YD), albumen length (AL), and albumen width (AW) were determined by using a digital caliper with  $\pm 0.01$  mm precision (Mitutoyo, 300 mm, Neuss, Germany) to calculate the yolk index (YI), albumen index (AI), and Haugh unit (HU). Albumen height (AH) and yolk height (YH) were measured by using a tripod micrometer. Egg yolk index, albumen index, and Haugh unit were calculated using the formulas given by Funk, Heiman and Carver, and Haugh [23,24], respectively:

$$YI = (YH/YD) \times 100$$

$$AI = (AH/(AL + AW)/2) \times 100$$

$$HU = 100 \times \log (AH + 7.57 - 1.7 \times EW^{0.37})$$

Yolk color was determined with a Roche yolk color fan with a 15-point scale (Roche Ltd., Basel, Switzerland), according to the pigmentation degree from the lightest (score 1) to the darkest color (score 15).

### Bone Characteristics

To evaluate the leg bone characteristics at 66 weeks of age, tibia and femur of both legs (including cartilage) were sampled from randomly selected ( $n = 15$  bone/genotypes) and euthanized by cervical dislocation. After dissection of tibia and femur, the samples were frozen at  $-20^\circ\text{C}$  until measurements.

After thawing process of bone samples, each bone was checked for any residue of soft tissues and then treated to drying at  $22^\circ\text{C}$  for 7 d. Then, the bone weight was measured with  $\pm 0.01$  g precision, and bone length and width (at 50% of the bone length) were measured by using a digital caliper. The relative weight of the tibia and femur was calculated as the ratio between bone weight and birds' weight. Then, relative asymmetry for bone length was calculated with the formula given by Møller et al. [25]:

$$RA = \{|R - L| / (R + L)/2\} \times 100$$

in which, RA means relative asymmetry of the left and right bone (%), R means length of the right bone (mm), L means length of the left bone (mm), and  $|R - L|$  means the absolute difference between R and L.

Breaking strength (N) for each tibia and femur samples was determined by a 3-point bending test using a fully computerized UTEST tensile and compression testing machine (Model 7014, UTEST Corp, Ankara, Türkiye) that was fitted with a 250 kN load cell. The crosshead movement was at 10 mm/min. The right tibia and femur were ashed, using AOAC method 932.16 [21].

The bone samples were subjected to a temperature of  $105^\circ\text{C}$  for 6 h and then defatted with hexane in a Soxhlet apparatus (Model SER148, Şimşek Laboratöknik, Ankara, Türkiye) for 4 h. After the extraction of fat, the bones were dried in a forced-ventilated oven at  $105^\circ\text{C}$  for 16 h to determine the dry and defatted weights of tibias. Then, the bone samples were crushed and calcined in a muffle furnace at  $600^\circ\text{C}$  for 2 h to determine the ash content.

### Statistical Analysis

The data on performance parameters (BW, EP, EM, DFI and FCR) for each genotype were analyzed by a one-way analysis of variance procedure (ANOVA) in the statistical analysis software SAS (version 9.4, 2012, Cary, NC, USA) [26]. A completely randomized design was used in the study. For laying performance, egg quality and bone

characteristics, the pens, eggs and, tibia and femur bones were respectively considered as the experimental unit. Significant differences between means were compared using the Tukey test. Analyses of percentage data were conducted after arcsine square root transformation of the data. The data are presented as LSmeans  $\pm$  SEM for each parameter. Differences were considered statistically significant at  $P < 0.05$ .

## RESULTS

The laying performance of commercial and Türk local laying hen genotypes in free-range system is given in Table 2. The mean of egg production showed a similar change between 54-57, 58-61 and 62-66 weeks of age. Lohmann Brown and Lohmann White had a higher egg production percentage during experimental period and varied between 85.6% and 86.5% in Lohmann Brown

and 86.5% to 87.6% in Lohmann White during three weeks periods from 54 to 66 weeks of age ( $P < 0.001$ ). During the experimental period, the eggs obtained from brown laying hen genotypes (Lohmann-Brown, Atak-S) tended to be heavier compared to the white genotypes (Lohmann-White, Atabey) ( $P < 0.001$ ). Between 54-57 weeks of age, the mean of egg weight varied from 61.3 g to 65.0 g, whereas the higher mean value of egg weight was observed in Lohmann Brown (65.5 g and 66.1 g) and Atak-S (65.4 g and 65.9 g) genotypes between 58-61 and 62-66 weeks of age respectively. A lower mean of egg mass was observed in Atak-S and Atabey genotypes ranged from 48.6 and 50.1 g, respectively, between 54-66 weeks of age ( $P < 0.001$ ). Similar changes were observed for egg mass during experimental period. White hen genotypes (Lohmann-White, Atabey) had a lower daily feed intake compared to both of brown hen genotypes (Lohmann-Brown, Atak-S). Lohmann Brown laying hens had the

**Table 2.** Laying performance of commercial and Türk local laying hen genotypes in free-range system ( $n = 3$  pens/genotypes)

Parameters	Genotypes	Age (weeks of age)			54-66
		54-57	58-61	62-66	
Egg production (%)	Lohmann Brown	86.5 $\pm$ 0.62 <sup>a</sup>	86.2 $\pm$ 0.80 <sup>a</sup>	85.6 $\pm$ 0.65 <sup>a</sup>	86.1 $\pm$ 0.65 <sup>a</sup>
	Lohmann White	87.6 $\pm$ 0.88 <sup>a</sup>	87.1 $\pm$ 0.72 <sup>a</sup>	86.5 $\pm$ 0.80 <sup>a</sup>	87.1 $\pm$ 0.80 <sup>a</sup>
	Atak-S	75.3 $\pm$ 0.83 <sup>c</sup>	74.8 $\pm$ 0.80 <sup>c</sup>	74.4 $\pm$ 0.72 <sup>c</sup>	74.8 $\pm$ 0.78 <sup>c</sup>
	Atabey	79.4 $\pm$ 0.58 <sup>b</sup>	78.8 $\pm$ 0.60 <sup>b</sup>	78.2 $\pm$ 0.52 <sup>b</sup>	78.8 $\pm$ 0.57 <sup>b</sup>
	<i>P value</i>	<0.001	<0.001	<0.001	<0.001
Egg weight (g)	Lohmann Brown	65.0 $\pm$ 0.25 <sup>a</sup>	65.5 $\pm$ 0.33 <sup>a</sup>	66.1 $\pm$ 0.12 <sup>a</sup>	65.5 $\pm$ 0.13 <sup>a</sup>
	Lohmann White	61.3 $\pm$ 0.09 <sup>c</sup>	61.7 $\pm$ 0.17 <sup>c</sup>	62.3 $\pm$ 0.13 <sup>c</sup>	61.8 $\pm$ 0.05 <sup>c</sup>
	Atak-S	64.5 $\pm$ 0.25 <sup>a</sup>	65.4 $\pm$ 0.14 <sup>a</sup>	65.9 $\pm$ 0.23 <sup>a</sup>	65.3 $\pm$ 0.15 <sup>a</sup>
	Atabey	62.9 $\pm$ 0.15 <sup>b</sup>	63.6 $\pm$ 0.27 <sup>b</sup>	64.0 $\pm$ 0.27 <sup>b</sup>	63.5 $\pm$ 0.16 <sup>b</sup>
	<i>P value</i>	0.001	<0.001	0.001	<0.001
Egg mass (g)	Lohmann Brown	56.2 $\pm$ 0.31 <sup>a</sup>	56.5 $\pm$ 0.25 <sup>a</sup>	56.6 $\pm$ 0.47 <sup>a</sup>	56.4 $\pm$ 0.34 <sup>a</sup>
	Lohmann White	53.7 $\pm$ 0.48 <sup>b</sup>	53.7 $\pm$ 0.37 <sup>b</sup>	53.9 $\pm$ 0.56 <sup>b</sup>	53.8 $\pm$ 0.46 <sup>b</sup>
	Atak-S	48.6 $\pm$ 0.62 <sup>c</sup>	49.0 $\pm$ 0.61 <sup>c</sup>	49.0 $\pm$ 0.62 <sup>c</sup>	48.8 $\pm$ 0.60 <sup>c</sup>
	Atabey	49.9 $\pm$ 0.48 <sup>c</sup>	50.1 $\pm$ 0.29 <sup>c</sup>	50.1 $\pm$ 0.47 <sup>c</sup>	50.0 $\pm$ 0.41 <sup>c</sup>
	<i>P value</i>	0.001	<0.001	<0.001	0.001
Daily feed intake (g)	Lohmann Brown	130.1 $\pm$ 1.39 <sup>a</sup>	130.7 $\pm$ 1.54 <sup>a</sup>	131.9 $\pm$ 1.18 <sup>a</sup>	130.9 $\pm$ 1.37 <sup>a</sup>
	Lohmann White	114.3 $\pm$ 0.97 <sup>c</sup>	116.0 $\pm$ 1.20 <sup>c</sup>	117.3 $\pm$ 1.30 <sup>c</sup>	115.8 $\pm$ 1.13 <sup>c</sup>
	Atak-S	121.2 $\pm$ 1.02 <sup>b</sup>	122.8 $\pm$ 1.16 <sup>b</sup>	124.4 $\pm$ 1.10 <sup>b</sup>	122.8 $\pm$ 1.06 <sup>b</sup>
	Atabey	115.0 $\pm$ 1.59 <sup>c</sup>	115.7 $\pm$ 1.45 <sup>c</sup>	116.4 $\pm$ 1.39 <sup>c</sup>	115.7 $\pm$ 1.30 <sup>c</sup>
	<i>P value</i>	0.001	<0.001	<0.001	<0.001
FCR (g feed/g product)	Lohmann Brown	2.31 $\pm$ 0.04 <sup>b</sup>	2.32 $\pm$ 0.03 <sup>b</sup>	2.33 $\pm$ 0.04 <sup>b</sup>	2.32 $\pm$ 0.04 <sup>b</sup>
	Lohmann White	2.13 $\pm$ 0.03 <sup>c</sup>	2.16 $\pm$ 0.04 <sup>c</sup>	2.18 $\pm$ 0.04 <sup>c</sup>	2.15 $\pm$ 0.03 <sup>c</sup>
	Atak-S	2.50 $\pm$ 0.04 <sup>a</sup>	2.51 $\pm$ 0.03 <sup>a</sup>	2.54 $\pm$ 0.02 <sup>a</sup>	2.52 $\pm$ 0.03 <sup>a</sup>
	Atabey	2.30 $\pm$ 0.04 <sup>b</sup>	2.31 $\pm$ 0.04 <sup>b</sup>	2.33 $\pm$ 0.05 <sup>b</sup>	2.31 $\pm$ 0.04 <sup>b</sup>
	<i>P value</i>	0.0007	0.0009	0.0016	0.0009

<sup>a-c</sup> values within columns with different superscripts are significantly different ( $P < 0.05$ )

**Table 3.** Egg characteristics of commercial and Türk local laying hen genotypes (n= 15 eggs/genotypes)

Parameters	Characteristics	Genotypes				P value
		Lohmann Brown	Lohmann White	Atak-S	Atabey	
Egg content	Egg weight (g)	65.3±0.05 <sup>a</sup>	61.5±0.07 <sup>c</sup>	65.1±0.05 <sup>a</sup>	63.9±0.09 <sup>b</sup>	<0.001
	Yolk (%)	20.1±0.05	19.0±0.68	17.6±0.64	20.6±0.09	0.235
	Albumen (%)	69.4±0.05	70.9±0.72	71.9±0.68	69.0±0.09	0.284
	Eggshell (%)	10.5±0.05	10.1±0.06	10.5±0.04	10.4±0.05	0.138
Exterior egg quality parameters	Egg shape index (%)	76.7±0.15	75.7±0.30	77.3±0.15	77.0±0.26	0.182
	Eggshell breaking strength (g/cm <sup>2</sup> )	3.350±0.02 <sup>a</sup>	2.847±0.03 <sup>d</sup>	3.300±0.03 <sup>b</sup>	2.910±0.02 <sup>c</sup>	<0.001
	Eggshell thickness (mm)	0.370±0.002	0.340±0.007	0.375±0.003	0.357±0.005	0.180
Interior egg quality parameters	Yolk index (%)	46.8±0.22	47.2±0.49	46.7±0.47	47.4±0.45	0.937
	Yolk color	12.3±0.15	11.7±0.15	12.3±0.15	12.3±0.15	0.441
	Albumen index (%)	11.6±0.28	11.9±0.15	10.7±0.24	11.7±0.25	0.440
	Haugh unit	89.2±0.70	89.3±0.96	88.6±1.35	89.9±1.04	0.983

<sup>a-d</sup> values within rows with different superscripts are significantly different (P<0.05)

**Table 4.** Morphological and mechanical traits of leg bones in commercial and Türk local laying hen genotypes at 66 weeks age (n=15 bones/genotypes)

Bone	Characteristics	Genotypes				P value
		Lohmann Brown	Lohmann White	Atak-S	Atabey	
Femur	Length (mm)	81.1±0.40 <sup>b</sup>	72.1±0.34 <sup>d</sup>	84.7±0.50 <sup>a</sup>	75.4±0.49 <sup>c</sup>	<0.001
	Width (mm)	16.3±0.21 <sup>a</sup>	11.5±0.18 <sup>c</sup>	13.9±0.11 <sup>b</sup>	12.4±0.15 <sup>c</sup>	0.001
	Weight (g)	11.4±0.15 <sup>a</sup>	7.4±0.18 <sup>b</sup>	12.1±0.10 <sup>a</sup>	8.2±0.12 <sup>b</sup>	0.001
	Relative weight (%)	0.58±0.008 <sup>a</sup>	0.44±0.01 <sup>b</sup>	0.55±0.005 <sup>a</sup>	0.52±0.007 <sup>a</sup>	<0.001
	Relative asymmetry (%)	0.278±0.03	0.219±0.04	0.290±0.03	0.358±0.05	0.559
	Breaking strength (N)	339.6±6.8 <sup>a</sup>	230.6±4.8 <sup>c</sup>	369.6±5.8 <sup>a</sup>	283.2±7.0 <sup>b</sup>	<0.001
	Ash (%)	57.9±0.76 <sup>ab</sup>	48.7±0.46 <sup>c</sup>	60.8±0.70 <sup>a</sup>	55.9±0.61 <sup>b</sup>	<0.001
Tibia	Length (mm)	109.2±0.57 <sup>b</sup>	110.2±0.59 <sup>b</sup>	118.7±0.98 <sup>a</sup>	109.9±0.69 <sup>b</sup>	0.001
	Width (mm)	15.4±0.19 <sup>a</sup>	11.4±0.20 <sup>b</sup>	14.7±0.20 <sup>a</sup>	12.6±0.15 <sup>b</sup>	<0.001
	Weight (g)	13.6±0.12 <sup>b</sup>	8.7±0.16 <sup>d</sup>	15.3±0.22 <sup>a</sup>	9.9±0.13 <sup>c</sup>	<0.001
	Relative weight (%)	0.68±0.005 <sup>b</sup>	0.52±0.01 <sup>c</sup>	0.69±0.01 <sup>a</sup>	0.63±0.008 <sup>b</sup>	<0.001
	Relative asymmetry (%)	0.169±0.02	0.216±0.03	0.131±0.01	0.264±0.05	0.318
	Breaking strength (N)	366.0±9.5 <sup>a</sup>	267.0±7.8 <sup>c</sup>	381.2±10.1 <sup>a</sup>	322.2±6.8 <sup>b</sup>	<0.001
	Ash (%)	55.5±0.61 <sup>a</sup>	48.2±0.53 <sup>c</sup>	53.2±0.51 <sup>a</sup>	51.8±0.46 <sup>b</sup>	<0.001

<sup>a-d</sup> values within rows with different superscripts are significantly different (P<0.05)

highest daily feed intake with values of 130.1g between 54-57 weeks of age, 130.7 g between 58-61 weeks of age, 131.9 g between 62-66 weeks of age (P<0.001). On the other hand, Lohmann White hens had a better FCR than the other white and brown genotypes (2.13, 2.16, 2.18, and 2.15 between 54-57, 58-61, 62-66 and 54-66 weeks of age respectively, P<0.001) (Table 2).

The egg characteristics of commercial and Türk local laying hen genotypes in free-range system is given in Table 3. The egg weight was found to be heavier in brown layer hen genotypes (Lohmann-Brown, Atak-S) (P<0.001),

whereas any significant differences was observed for the percentage of yolk, albumen and eggshell among the brown and white genotypes (Lohmann-White, Atabey) (P>0.05). A higher mean value of eggshell breaking strength was found in brown eggs (3.350 g/cm<sup>2</sup> and 3.300 g/cm<sup>2</sup>) than white eggs (2.847 g/cm<sup>2</sup> and 2.910 g/cm<sup>2</sup>, P<0.001). However, egg shape index, eggshell thickness and interior egg quality parameters was found to be similar among the brown and white hen genotypes (P>0.05) (Table 3).

The morphological and mechanical traits of femur and tibia bones of commercial and Türk local laying hen

genotypes in free-range system is given in *Table 4*. The femur length was found to be the highest in Atak-S laying hen genotypes, whereas the femur width was the highest in Lohmann Brown hen genotype ( $P < 0.001$ ). A higher weight of femur was observed in brown hen genotypes (Lohmann-Brown, Atak-S), while the lowest value of femur relative weight with a value of 0.44% was found to be in Lohmann White laying hens ( $P < 0.001$ ). On the other hand, the breaking strength and ash content of femur was the lowest in Lohmann White laying hens than the other hen genotypes (230.6 N and 48.7%,  $P < 0.001$ ). The tibia was observed as the longest in Atak-S laying hens (118.7 mm,  $P < 0.01$ ). The width of tibia was found to be higher in brown laying hen genotypes (Lohmann-Brown, Atak-S) compared to the white laying hen genotypes (Lohmann-White, Atabey) ( $P < 0.001$ ). On the other hand, the weight and relative weight of tibia were the highest in Atak-S laying hens (15.3 g and 0.69%,  $P < 0.001$ ). A higher mean value of breaking strength and ash content was observed in brown laying hen genotypes (Lohmann-Brown, Atak-S) than the white laying hen genotypes (Lohmann-White, Atabey) ( $P < 0.001$ ) (*Table 4*).

## DISCUSSION

The current study clearly indicated significant differences for productivity between commercial (Lohmann-Brown, Lohmann-White) and Türk laying hen genotypes (Atak-S, Atabey) kept in the free-range system. Küçükyılmaz et al.<sup>[27]</sup> reported a higher production rate for white layer hen genotype than the brown one in both organic production (87.23% vs. 82.50%) and conventional (89.82% vs. 80.43%) system between 23 and 70 weeks of age. On the other hand, brown eggs tended to be heavier compared to the white eggs, whereas eggs obtained from Atak-S and Lohmann White genotypes had a lower egg mass. This could be originated from observed differences for egg production rate and also egg weight.

Sozcu et al.<sup>[28]</sup> study showed that Atabey hens (75.9%) had a higher egg production level than Atak-S hens in a free range system (70.3%). Otherside, Atak-S hens tended to consume more feed than Atabey hens. Daily feed intake was found to be higher in Atak-S (117.2 g) than in Atabey (109.8 g). Higher FCR between 19 and 72 weeks of age was observed in Atabey than in Atak-S (2.48 vs. 2.54).

There are other contradictory results for differences between layer hen genotypes in free-range system. Küçükyılmaz et al.<sup>[27]</sup> and Rizzi and Chiericato<sup>[29]</sup> demonstrated that commercial hybrids had a higher egg production rate and egg mass than native hybrids. On the other hand, Şekeroğlu and Sarıca<sup>[30]</sup> found a higher egg production rate in native hybrids in free-range system.

The feed intake and FCR were significantly differed

among the genotypes in the study. The brown layer hens consumed more feed than white hens and had a worse FCR value. Observed higher feed intake could be attributed to the a higher egg weight in brown eggs, which resulted in increment of feed intake due to a higher energy requirement. On the other hand, the hens had free access to pasture area, therefore possible to more physical activity, such as walking and foraging behaviors, which also increased the energy requirement of laying hens. Küçükyılmaz et al.<sup>[27]</sup> emphasized that brown layers were more active with scratching and foraging behaviors than white hens in the organic production system and found similar feed intake for brown and white layer hens (Atak-S and Lohmann White) (127.74 g and 127.69 g). These findings are consistent with previous reports by Lampkin<sup>[31]</sup> and Castellini et al.<sup>[32]</sup>.

Egg exterior and interior characteristics, have importance during commercial handling and transport processes and also consumer preference<sup>[33]</sup>. In the present study, only eggshell breaking strength showed difference between hen genotypes. Brown eggs had a stronger shell structure with a higher breaking strength value compared to the white eggs. Otherwise, Atak-S Brown hen genotype had higher shape index (77.9%), eggshell breaking strength (3.429 g/cm<sup>2</sup>), shell thickness (0.371 mm) than Atabey white hen genotype (76.0%, 2.982 g/cm<sup>2</sup>, 0.361 mm) respectively in another study<sup>[28]</sup>.

The observed differences could be attributed to both the genetical differences and more motor activity in pasture. Thus, brown hens could have more time on pasture which provide more ingestion of tiny stone and longer exposure duration to sunlight which promote mineral metabolism. This could lead more accumulation of minerals in the shell, and subsequently more stronger shell structure in brown eggs<sup>[34]</sup>.

The skeleton of birds take parts in eggshell formation (up to 40%) by providing calcium<sup>[35,36]</sup>. High calcium requirements for shell formation could cause an increment bone mineral resorption from medullary bone. This is the main cause of osteoporosis, and this could mainly develop from age and low calcium content of diet<sup>[37,38]</sup>. According to these facts, it could cause a negative relationship between egg production and skeletal integrity in layer hens<sup>[39,40]</sup>. In previous studies, significant negative correlation was found between eggshell quality (shell weight) and bone mineralization (bone ash content). It could be explained that laying hens are more prone to osteoporosis due to mobilizing more calcium for better quality of shell. Furthermore, some studies reported any significant relationship between bone quality, egg production and shell quality<sup>[41,42]</sup>.

Interestingly, current findings demonstrated that femur

and tibia characteristics namely length, width, weight, breaking strength and ash content had higher mean values in brown layer hens compared to the white layer hens. A previous study reported a negative correlation between shell breaking strength and ash content of bones [43]. It could be attributed to more physical activity at outdoor area and directly exposure to sun light of brown layer hens. In this study, both of brown genotypes had more time in pasture area. It is well known that range usage in free range system provides daylight to hens and it stimulates hormone and vitamin D production which could improve bone mineralisation [44].

In a recent study performed by Alfonso-Carrillo et al. [45], it was reported that hens with higher egg production rate and good shell quality had higher body weight and slightly larger uterus (shell gland) compared to the other groups with lower production and poorer shell quality. Therefore, hens with higher production with good quality of shell had a greater capacity to mobilize calcium for shell formation and retained lower amount of medullary bone. In current study, brown laying hens from Lohmann Brown and Atak-S genotypes (1982.1 g and 2209.6 g respectively) had higher body weights than Lohmann White and Atabey white genotypes (1678.5 g and 1575.7 g respectively) at 66 weeks of age ( $P=0.001$ ).

In conclusion, current data demonstrated the differences between commercial hybrids and Türk genotypes kept in a free-range system between 54-66 weeks of age. These findings clearly showed that white layer hens had a higher productivity level with better FCR, whereas the brown layer hens had a superiority for egg weight, shell strength and bone integrity. To decide the production target between productivity versus welfare, it is important to remember that it is aimed to improve health and welfare status in the free-range systems. As well as priority preference of native genotypes in the free-range system, Atak-S could be preferred to produce heavier eggs with stronger shell structure and bone traits, whereas Atabey could be chosen for a higher egg production and better feed utilization.

#### Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author (A. Sözcü). The data are not publicly available due to privacy or ethical restrictions.

#### Ethical Approval

The care and use of animals has been approved by the ethics committee of Bursa Uludağ University and comply with Türkiye laws and regulations (License Number 2019-05/09).

#### Financial Support

The authors has not been received any funding for this work.

#### Conflict of Interest Statement

The authors declared that there is no conflict of interest.

#### Author Contributions

Methodology and collection of data, A.I, A.S., investigation and data analysis, A.S., M.G., writing - review and editing A.S., A.I., M. G. All authors have read and agreed to the published version of the manuscript.

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