## **Research Article**

# Comparative Evaluation of Table Egg Quality of Local and Pure Breed Laying Hens in Response to Storage Period Length

Metin PETEK <sup>1</sup> <sup>(\*)</sup> <sup>(\*)</sup> Tuğba KAHRAMAN <sup>1</sup>

<sup>1</sup>Bursa Uludag University, Faculty of Veterinary Medicine, Department of Animal Science, TR-16059 Bursa - TÜRKİYE



 <sup>(+)</sup> Corresponding authors: Metin PETEK
 Phone: +90 224 294 1352; Cellular phone: +90 535 770 5994; Fax: +90 224 2941200

E-mail: petek@uludag.edu.tr

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#### Abstract

This study was made to investigate the fresh and stored egg quality characteristics of local and pure-breed layer chickens. The eggs were randomly collected from a commercial farm that raised different free-range layer flocks such as local Atak-S, commercial Nick Brown, pure-breed Sussex laying hen, and a local cross-breed hen, all of 50 weeks of age. In total, 240 eggs, 60 eggs from each genotype, were further divided into three groups as: fresh eggs, 15 and 28-day storage period, and were stored at 14-16°C and 45-50% humidity conditions. The eggs were analyzed on the basis of internal and external quality, such as egg length, egg width, color characteristics, yolk height, albumin height, yolk color, and Haugh unit. Genotype had a significant effect on shell weight (P<0.001), shell thickness (P<0.001), albumen index (P<0.001), yolk index (P<0.001), and HU value (P<0.003). The effects of the storage period on albumen index, yolk index, and HU value were found to be significant, respectively (P<0.001, P<0.001, P<0.001). Significant differences existed for the shape index, L\*, a\*, b\*, E, and C\* values among the genotype groups (P<0.001). In conclusion, eggs of the local, pure-breed, and cross-breed layer chickens showed differences from the eggs of commercial hybrid hens both for external and internal quality. Eggs of commercial Nick Brown and Sussex breed seems slightly better in longer storage conditions.

Keywords: Atak-S, Sussex, Free-range, Storage period, Egg quality

# INTRODUCTION

Poultry eggs are the most economical source of animal protein and calories for people across the world. Protecting the quality, freshness, and nutritious value of eggs throughout the whole marketing chain is very crucial to human nutrition and food security. The egg quality of poultry is influenced by both genetic and environmental factors such as storage length, animal feed, storage temperature, housing condition <sup>[1-4]</sup>, etc. Good management practices of environmental factors and layer genotypes, such as pure breed or local poultry, can help prevent egg quality, such as shell color, egg weight, shell weight, and yolk weight <sup>[5]</sup>.

Commercial layer genotypes have been selected for generations according to their production performance and quality of eggs, especially for cage housing <sup>[6]</sup>. In recent years, consumer interest in eggs from natural or backyard systems has led to a constantly decreasing trend in the number of hens housed in the conventional cage system in favor of non-caged or free-range housing systems. But

all commercial chickens are not entirely convenient for cage-free or free-range egg production, so using pure or local poultry breeds in these systems is becoming popular for table egg production at first <sup>[7]</sup>. Consequently, some commercial layers were developed for free-range egg production <sup>[8]</sup>. As a result of consumer interest in native product, local layer or pure breed hens have been of interest most because they can efficiently produce eggs under adverse environmental conditions and contribute to prevent the animal welfare and biodiversity <sup>[9]</sup>. Higher interest of consumers for eggs produced in non-cage systems and some welfare problems in these housing systems, leading to an increase in the need for information to find out the best genotype for non-cage free-range systems. Knowledge on the egg quality of pure-bred or local hens during the productive period is required for sustainable egg production.

In practice, it is required to store table eggs less or more according to marketing conditions. During this stage, protecting the freshness and quality of eggs is of utmost

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importance. Some components of albumen and yolk may alter and tend to deteriorate egg quality during egg storage. The main factors directly associated with egg deterioration are temperature and relative humidity conditions, besides egg handling and storage time affecting egg quality and shelf life <sup>[10]</sup>. The higher the storage temperature, the faster the egg quality decreases. Egg weight, albumen height, and HU decreased significantly, while albumen pH increased with the extension of storage time <sup>[11]</sup>. The storage method had a significant effect on most of the egg quality traits, and eggs stored at 4°C were of good quality and were classified as extra-class eggs even after 28 days [12]. But in most cases, eggs stored in non-refrigerated conditions and the deterioration rate of different kinds of poultry eggs might be different due to their egg quality properties. It was reported that eggs stored at room temperature should be consumed in 2 weeks or refrigerated until eight weeks [13]. According to the Turkish Food Codex on Egg and Egg Products <sup>[14]</sup>, it is not necessary to cool the egg until the 18th day after the ovulation date. However, and then, it should be stored between +8 and +5°C starting from the 18th day. In Türkiye, 95.7% of all table eggs are produced in closed barn systems as conventional cages, unfurnished cages, furnished cages, and non-cage systems, while 4.3% of all table eggs are produced in organic and non-organic free-range conditions [15].

Currently, there has been growing consumer interest in purchasing eggs produced by local or pure-breed hens such as Atak-S and Sussex kept in closed barns and extensive rearing systems <sup>[16,17]</sup>. In general, local or pure-breed layers are characterized by a small number of egg production, resulting in a lower profit for producers and a higher cost of eggs for the consumer compared to conventional eggs produced by commercial genotypes [18]. However, the benefits of raising these genotypes for egg production have not been assessed in terms of consumer expectations such as egg quality, food safety, economic sustainability, welfare, and the environment. It is not clear whether the egg quality of local or pure-breed chicken can keep up with that of commercial laying hens. Because consumers increasingly pay attention to not only egg weight and shell quality but also their taste, freshness, yolk and shell color, nutritional value, etc. There is also a need to investigate these layer genotypes' internal and external egg quality characteristics in different storage conditions. Physical egg quality parameters, such as shell strength, shell color, Haugh unit, and yolk color, can affect consumer perceptions and might be different. Therefore, this study was conducted to investigate the effects of the length of storage period on the physical quality characteristics of eggs of different genotypes of layer chickens as local, pure, and cross-breed hens compared to commercial laying hens.

# MATERIAL AND METHODS

#### **Ethical Statement**

This study does not require ethical permission.

#### Location

Each flock was housed on the same farm separately in Bursa, Türkiye, through the laying period according to standard procedures and Turkish legislation to protect laying hens <sup>[19,20]</sup>.

#### **Animals and Eggs**

The eggs used in this study were collected from a commercial farm raised four in different layer flocks: commercial Nick Brown, pure-breed Sussex laying hen, local Atak-S, and a local cross-breed hen. Atak-S hens were developed by the Republic of Türkiye Ministry of Agriculture and Forestry and have been raised commonly in free-range egg production in Türkiye <sup>[21]</sup>. The cross-breed hens originated from Araucana chickens and produced a mix color of blue, green, and white eggs. Sussex and Atak-S hens are representative of layer hens laying brown eggs that range from cream to light brown in color. Eggs from the commercial Nick Brown are completely dark brown. The eggshell of the cross-breed had a green/blue surface on the eggs (*Fig. 1*).



Fig 1. Shell color variations of eggs of different genotype of layer chickens

## Management

A standard layer diet for the hens was used between 22 and 45 weeks of age (17.86% crude protein, 2.750 metabolizable energy kcal/kg). Subsequently, a second-phase diet was used between 45 and the end of the laying period (16.45% CP, 2800 ME kcal/kg). Hens were housed in a closed

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deep litter barn with no windows and free access to the open range area. Less than 30% of the outdoor condition was covered by natural grass. The stocking density was nine hens per m<sup>2</sup>, and 250 cm<sup>2</sup> of litter area in a closed barn, and 4 m<sup>2</sup> per hen on the range area, as stated in the Turkish legislation for the protection of laying hens <sup>[21]</sup>. All houses were equipped with perches, a feeding trough, and a nipple for water supply. Each house had individual nest boxes (1 nest box per 8 hens). Nest space and perch length (15 cm per hen) per hen followed the Turkish legislation for the protection of laying regime was 16 h per 24 h period from 28 weeks to 72 weeks of age.

#### Sampling/Data Collection

To evaluate the physical characteristics, eggs were collected from layers of each genotype and divided into three groups: fresh, 15-days storage lengths, and 28-days storage lengths. Fresh eggs were evaluated after 24 h of storage at around 14-16°C and 45-50% humidity. This temperature interval corresponds to the EU regulation EC 589/2008 [8,22] and Turkish regulation [14], since it advises consumers to keep eggs refrigerated after purchase. Eggs in the 15<sup>th</sup> and 28<sup>th</sup> days of storage period groups were stored at similar conditions until analysis. Egg analysis was carried out in the Egg Quality Laboratory of the Department of Animal Science at Faculty of Veterinary Medicine, Bursa Uludag University. The egg quality evaluation included egg weight, egg length, egg width, shell color (L\*, a\*, b\*) traits, shell thickness, shell weight, yolk height, thick albumen height, and yolk color. Egg shell color characteristics, egg width, and egg length were measured only on all fresh eggs collected from the flocks, while shell weight, shell thickness, and other internal quality characteristics were measured every period.

In total, 60 eggs from a whole day's production per each genotype were randomly collected, excluding the defective eggs as double-yolk, without shell. Eggs in all groups were weighed with a precision digital scale (0.01 precision). After weighing, the width (along the equatorial axis) and length (along the longitudinal axis) of the eggs were measured with a caliper to 0.1 mm. The egg shape index was calculated as egg width/egg length x 100. The shell colour was measured by a colorimeter (PCE-XXM 20, PCE Instruments Ltd) using the CIE L\*a\*b\*scale <sup>[23]</sup>. The calorimeter was calibrated on a predefined white plate under daylight <sup>[24]</sup>.

L\*-lightness (ranges from 0 for an extremely black and to 100 for a perfectly white);

a\*-chromaticity in the red-green axis (red; if it is positive up to 100, green; if it is negative up to -100);

b\*-chromaticity in the yellow-blue axis (yellow; if it is positive up to 100, blue; if it is negative up to -100);

C-chroma (the distance of the color point to the L\*-axis);

E value is calculated as the square root of the sum of the squares of L\*, a\* and b\* values. The color intensity/ saturation index (chroma value) is the square root of the sum of the squares of a\* and b\* values  $^{[25-27]}$ .

After the completed measurement of external quality traits, the eggshell was broken along the equatorial axis, and the yolk and albumen were put on a flat glass plate surface. The height of thick albumen and yolk height were measured using a tripod micrometer (Mitutoya, 200 mm). The albumen length and albumen width were determined by using a digital caliper with  $\pm$  0.01 mm precision (Mitutoyo, 300 mm, Neuss, Germany). The color intensity of the yolk was determined by visual comparison to a yolk color fan <sup>[28]</sup>. Colour scales ranged from 1 (pale yellow) to 15 (intense orange). The yolk was manually separated from the albumen and weighed. The albumen weight was calculated as the difference between the egg weight and the sum of shell weight and yolk weight. To measure shell thickness, pieces from three different points of each eggshell with intact membranes were measured with a micrometer to 0.01 mm. The egg shells were washed with water and set to dry at room temperature for 48 h, after which the shell weight was recorded. The egg yolk index, albumen index, and Haugh unit were calculated using the formulas given by, DSM egg quality manual <sup>[28]</sup>, Funk <sup>[29]</sup>, Bender <sup>[30]</sup>, and Haugh <sup>[31]</sup>, espectively.

$$Yolk \ Index = \frac{Yolk \ Height}{Yolk \ width} \ x \ 100$$

$$Albumen \ Index = \frac{Thick \ Albumen \ Height}{Thick \ Albumen \ Width + Thick \ Albumen \ Length/2} \ x \ 100$$

 $Haugh Unit = 100 Log (Albumen Height + 7.57) - (1.7xEgg Weight^{0.37})$  where, albumen height (H) in mm, egg weight (W) in grams.

#### **Statistical Tests**

The collected data were statistically analyzed using SPSS 24.0 statistical package <sup>[32]</sup>. The effects of genotype on shape index and egg color traits were tested using one-way analysis of variance. The significance of differences between the average values of breeds and storage length for all other traits was evaluated using two-way analysis of variance (ANOVA) with Tukey's post-hoc test <sup>[33]</sup>. In all statistical tests used, differences at P<0.05 were considered as significant.

### RESULTS

External quality traits of fresh eggs in different genotypes of layer chickens are presented in *Table 1*. There were significant differences for all external quality parameters of the eggs from different genotypes as shape index,  $L^*$ ,  $a^*$ ,  $b^*$ , E and Chroma values, respectively (P<0.001).

Egg weight, egg shell weight, and egg shell thickness values in the groups were shown in *Table 2*. There were

Table 1. External egg quality characteristics of daily eggs obtained from different genotype of layer chickens (Mean ± SEM)								
Genotype	Shape Index	L*	a*	b*	$\Delta E^*$	C*		
Atak-S	$73.96 \pm 0.58^{bc}$	$68.15 \pm 1.0^{b}$	32.10±1.45 <sup>a</sup>	20.43±0.76 <sup>b</sup>	86.55±1.33 <sup>b</sup>	52.71±1.64ª		
Nick Brown	76.78±0.35ª	49.92±0.17°	48.38±1.67 <sup>b</sup>	34.83±1.34ª	69.47±0.88°	48.12±1.19ª		
Sussex	$75.09 \pm 0.42^{ab}$	71.93±1.48 <sup>b</sup>	35.36±2.61 <sup>b</sup>	20.36±0.74 <sup>b</sup>	84.11±1.18 <sup>b</sup>	41.55±2.28 <sup>b</sup>		
Cross Breed	73.19±0.62°	93.16±0.67ª	-25.77±1.91°	7.96±0.42°	97.60±0.39ª	27.26±1.82°		
P-value	0.001	0.001	0.001	0.001	0.001	0.001		

 $L^*$ : Brightness,  $a^*$ : redness,  $b^*$ : yellowness,  $\Delta E^*$ : color difference,  $C^*$ : color saturation index

a-c: Different letters within the columns indicate significantly important differences

Groups		Egg Weight (g)	Shell Weight (g)	Shell Thickness (µm)	
Genotype (G)	Atak-S	62.45±0.90	5.96±0.12 <sup>b</sup>	0.34±0.007 <sup>ab</sup>	
	Nick Brown	61.12±0.89	7.12±0.14ª	0.36±0.006ª	
	Sussex	62.59±0.91	5.81±0.13 <sup>b</sup>	0.32±0.007 <sup>b</sup>	
	Cross Breed	61.01±0.89	5.23±0.14°	0.33±0.006 <sup>b</sup>	
Storage Period (SP)	0	65.41±0.67ª	6.14±0.10	0.36±0.005ª	
	15	60.69±0.67 <sup>b</sup>	5.94±0.10	0.34±0.005 <sup>b</sup>	
	8	59.28±0.95 <sup>b</sup>	5.97±0.14	0.30±0.007°	
	A-0	65.31±1.34	5.94±0.20	0.35±0.010	
	A-15	61.30±1.34	5.97±0.20	0.35±0.010	
	A-28	60.74±1.89	5.97±0.29	0.31±0.014	
	NB-0	62.06±1.34	6.94±0.20	0.41±0.010	
	NB-15	60.98±1.34	7.11±0.20	0.33±0.010	
Genotype x	NB-28	60.34±1.89	7.52±0.29	0.32±0.014	
Storage Period	S-0	68.02±1.34	6.14±0.20	0.34±0.010	
	S-15	60.59±1.34	5.23±0.20	0.32±0.010	
	S-28	59.16±1.89	6.31±0.29	0.31±0.014	
	CB-0	66.25±1.34	5.56±0.20	0.34±0.010	
	CB-15	59.90±1.34	5.48±0.20	0.34±0.010	
	CB-28	56.88±1.89	4.08±0.29	0.27±0.014	
	G	n.s	0.001	0.001	
ANOVA	SP	0.001	n.s	0.001	
	GxSP	n.s	0.002	n.s	

*a-c:* Different letters within the same columns (genotype and storage period) indicate different values

no significant differences for the average egg weight of different layer genotypes while storage length had a significant effect on the egg weight of the laying hens (P<0.001). Genotype had a significant effect on egg shell weight (P<0.001) and shell thickness (P<0.001) while the effects of storage length on shell thickness were found to be significantly important (P<0.001). Genotype x storage length interaction for shell weight was found to be significantly important, as well. The effects of genotype and length of storage on internal egg quality traits were presented in *Table 3*. Genotype and length of storage had a significant effect on albumen weight (P<0.05), albumen index (P<0.001 and P<0.001), yolk index (P<0.001 and P<0.001) and HU (P<0.003 and P<0.001). Yolk weight was affected by genotype significantly (P<0.05). Genotype x storage length interaction for all internal parameters was found to be non-significantly important.

Groups		Albumen Weight	Yolk Weight	Albumen	Yolk	Yolk	HU
		(g)	(g)	Index	Index	Color	но
Genotype	Atak-S	38.86±0.56 <sup>ab</sup>	$17.63 \pm 0.39^{a}$	6.44±0.37 <sup>b</sup>	39.83±0.55 <sup>b</sup>	10.43±0.29	73.73±1.65 <sup>b</sup>
	Nick Brown	37.90±0.55 <sup>bc</sup>	16.10±0.43°	10.01±0.36ª	$42.40 \pm 0.53^{a}$	10.77±0.28	81.09±1.66ª
	Sussex	39.19±0.52ª	$17.59 \pm 0.44^{a}$	6.18±0.37 <sup>b</sup>	38.65±0.55 <sup>b</sup>	10.57±0.27	75.51±1.65 <sup>ab</sup>
	Cross Breed	37.24±0.50°	18.54±0.41ª	5.65±0.38 <sup>b</sup>	$40.37 \pm 0.54^{b}$	10.07±0.28	71.97±1.66 <sup>b</sup>
Storage Period	0	41.63±0.62ª	17.64±0.36	10.74±0.28ª	45.24±0.41ª	$10,88 \pm 0,21$	88.23±1.25ª
	15	37.39±0.61 <sup>b</sup>	17.36±0.38	5.05±0.28 <sup>b</sup>	38.50±0.41 <sup>b</sup>	10.30±0.21	70.76±1.25 <sup>b</sup>
	28	35.89±0.60 <sup>b</sup>	17.42±0.39	$4.02 \pm 0.40^{b}$	34.09±0.59°	10.20±0.30	59.95±1.76°
Genotype x Storage Period	A-0	41.89±1.09	$17.48 \pm 0.60$	9.26±0.56	45.20±0.83	$11.00 \pm 0.42$	87.52±2.49
	A-15	37.73±0.90	17.60±0.59	4.62±0.56	37.24±0.83	$10.90 \pm 0.42$	68.29±2.49
	A-28	36.96±0.89	17.81±0.61	5.43±0.79	34.24±1.17	9.40±0.59	57.05±3.53
	NB-0	39.19±0.91	15.93±0.59	8.00±0.56	47.73±0.83	$10.70 \pm 0.42$	97.07±2,49
	NB-15	37.97±1.01	$15.90 \pm 0.54$	5.36±0.56	40.03±0.83	$10.40 \pm 0.42$	74.57±2.49
	NB-28	36.35±0.99	16.47±0.58	3.34±0.79	36.47±1.17	11.20±0.59	62.20±3.53
	S-0	43.72±0.98	18.16±0.56	7.57±0.56	42.63±0.83	$10.80 \pm 0.42$	83.09±2.49
	S-15	37.76±0.92	17.60±0.59	5.81±0.56	37.60±0.83	$10.10 \pm 0.42$	73.84±2.49
	S-28	35.82±0.93	17.03±0.58	4.11±0.79	32.77±1.17	10.80±0.59	63.74±3.53
	CB-0	41.69±0.88	19.00±0.61	8.13±0.56	45.38±0.83	$11.00 \pm 0.42$	85.23±2.49
	CB-15	35.58±0.88	18.34±0.62	4.41±0.56	39.10±0.83	9.80±0.42	66.34±2.49
	CB-28	34.50±0.99	18.30±0.62	3.18±0.79	32.86±1.17	9.40±0.59	56.79±3.53
ANOVA	G	0.05	0.05	0.001	0.001	n.s	0.003
	SP	0.05	n.s	0.001	0.001	0.08	0.001
	GxSP	n.s	n.s	n.s	n.s	n.s	n.s

G: Genotype, SP: Storage Period, A: Atak-S, NB: Nick Brown, S: Sussex, CB: Cros-breed

a-c: Different letters within the same columns (genotype and storage period) indicate different values

# DISCUSSION

Egg weight is an essential trait for egg quality, and it mainly depends on the hen genotype. In general, native poultry breeds lay smaller eggs than commercial hybrid strains <sup>[34,35]</sup>. Local and exotic hens had smaller eggs than commercial hybrid hens [36]. Sözcü et al.[37] compared the performance of two Turkish local laying hens, and they reported that the eggs from Turkish Atak-S hens tended to be heavier than eggs from Turkish Atabey hens. Dual-purpose hens, which are used for both egglaying and meat, have lower quality eggs and egg weights if we compare them to commercial layer hens <sup>[38]</sup>. In this current study, there were no significant differences in the daily fresh egg weight of different genotypes. According to EU Commission regulations <sup>[22]</sup> eggs of all genotypes used in this study can be classified as medium eggs and their weight is found to be very close to the bottom level of large eggs (large weight  $\geq$ 63 g). This was important because egg quality may be negatively affected by higher egg weight due to the synthesis of calcium for eggshell mass and the

synthesis of protein in egg albumen. Weight loss during egg storage progressively increased with the length of the storage period. But the most distinctive losses were determined during the 15-day storage period. There were no significant differences for the egg weight between 15 and 28 days of the storage period. Along with the length of the storage period, egg weight loss can be affected by hen breed, breeder's age, room temperature, and room humidity etc.<sup>[39,40]</sup>.

There were significant differences in the shell weight of the eggs among the genotype groups. Commercial Nick Brown had significantly greater egg shell weight than the other genotype groups. Genotype x storage period interaction for eggshell weight revealed that length of storage had a significant effect on eggs of Sussex and local cross-breed hens, while no significant differences were detected for eggs of Atak-S and Nick Brown hens. This means egg loss in Nick Brown and Atak-S was found to be lower than Susses and Cross-breed hens with the length of egg storage. In this study, the eggs of commercial Nick Brown hen had significantly the greatest shape index value. Eggs of Sussex layer breed and Atak-S genotypes had similar shape index values. The shape index value of all breeds was between acceptable values of 72-76 [41] for A-grade eggs and eggs of Nick Brown was a bit higher than the acceptable standards. Although there was not much more impact of shape index on consumer interest in table eggs, the unnatural shape and poor shell quality of breeder hen eggs are not desired because of the higher risk of cracked eggs and poor hatchability [42]. In a study, it was reported that Italian dual-purpose purebred Ermellinata di Rovigo showed the lowest shape index compared to commercial layer hens [43]. In general, egg shell weight does not increase after 50 weeks of age while egg weight still increases after this age. This disbalance between egg and shell weight development results in an increased risk of weak shell quality and more broken/cracked eggs.

The most visible feature of table eggs and one of the most sensitive issues for consumers is their eggshell color and thickness which is an indicator of the shell quality of eggs. In this study, the shell thickness of the eggs was significantly affected by genotype and storage period (P<0.001). The shell thickness value of eggs in commercial Nick Brown and fresh eggs was found to be greater than the others. In agreement with our results, Grasshorn et al.<sup>[10]</sup> reported that eggshell thickness was negatively influenced by storage duration. Sözcü et al.<sup>[37]</sup> reported that Turkish Atak-S hens had a stronger eggshell structure than the eggs of Turkish Atabey hens. The shell thickness of eggs is mainly determined by the proportion of calcium deposition and the length of egg shell formation in the uterus <sup>[6]</sup>. Time spend in uterus and oviposition time also has an effect on the thickness of the eggshell. We did not use the same eggs in every period of storage to determine the egg quality. Individual differences for egg shell thickness might be a reason for differences in terms of egg storage.

Although eggshell color does not affect the shell quality, flavor, cooking characteristics, nutritional value, or shell thickness of eggs in general, especially in pure breed poultry, the eggshell color is characteristic of a specific poultry breed <sup>[44]</sup>. In this study, the eggshell colors of the hens were naturally different from each other (Fig. 1). The color variability of table eggs, brown and white, is attractive for consumers, particularly if the differences are as extreme as green or blue [45]. The cross-breed hens have a perfectly white eggshell color with a 93.16 L\* value. Atak-S and Nick Brown followed the cross-breed hen for the L\* value. Commercial Nick Brown that normally laying a dark brown egg had the lowest egg shell color lightness. It was found that the L\* values of the eggs in all breeds were in agreement with their eggshell color as darker or lighter. Like in an agreement with eggshell color, the "a" values of eggs from Nick Brown were found to be darker (redness) than the other genotypes. Eggs of cross-line hens had significantly more extreme a\* color trait values than the other eggs. As with other color characteristics, the E value in eggs of cross-line hens and the chroma values in eggs of Atak-S hens and Nick Brown hens were found to be significantly greater. Sussex püre-breed and Atak-S genotype had similar egg color characteristics as L\*, b\*, E, and chroma color values. Drabik et al.<sup>[44]</sup> showed that the proportion of particular mineral elements in eggs was correlated with the L\*, a\*, and b\* color space coordinates of egg shells. In that study, it was reported that there was a relationship between shell color and egg albumen quality. The color of eggshell is a result of pigments deposited on the shell of hens and L\*, a\*, b\* values of shell colour were varously correlated with pigment contents <sup>[46]</sup>.

In this study, layer genotype has significantly affected both the albumen weight and yolk weight of the eggs (P<0.05). Similarly, Nolte et al.<sup>[2]</sup> reported that the yolk and albumen percentages of the eggs were significantly influenced by the layer genotype. The eggs of Sussex pure-breed had the greatest albumen weight value, while the eggs of cross-breed hens had the largest yolk weight. Several authors reported that native breeds lay eggs with a higher percentage of yolk and a larger amount of albumen than commercial hybrid strains <sup>[34,47,48]</sup>. In a study, the albumen weight of the eggs of two commercial layer chickens (Hy-line white and brown) was found to be significantly greater than that of native Ermellinata di Rovigo and Robusta Maculata hens [35]. The albumen weight of the eggs was also significantly affected by the length of storage (P<0.05), while the albumen index of the eggs was significantly affected by both genotype and storage period (P<0.001). In agreement with our study, Kralik et al.<sup>[49]</sup> reported that the storage period significantly influenced the albumen height of the table eggs. A higher albumen height of eggs indicates denser albumen, and it directly increases the value of the Haugh unit.

Yolk color and yolk weight are commonly used for comparison to yolk quality. In this study, yolk weight and yolk index were significantly affected by genotype, while the storage period affected only the yolk index of the eggs. Similar to the findings of Giampietro-Ganeco et al.<sup>[50]</sup> the yolk index decreased with increasing storage time. The yolk index value of the fresh eggs should meet the standard reference value as a good yolk index of 0.45 reported by Mertens et al.<sup>[51]</sup>. According to the DSM egg quality manual, we can consider all eggs as fresh because eggs with a yolk index between 0.29 and 0.38 are identified as fresh <sup>[28]</sup>. But the eggs with prolonged storage periods and in all genotype groups were under the standard reference value for yolk index as extra fresh eggs (0.38). The eggs stored at room temperature might have been showing significant differences in the yolk index value

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from the 2nd week on <sup>[13]</sup>. A freshly laid egg yolk is round and firm. The strength of the yolk membrane weakens with yolk ages and allowing water to be absorbed from the white. In our study, there were no significant differences in the yolk color of eggs in all genotype and storage period groups. But Nolte et al. <sup>[2]</sup> reported that the yolk color of the eggs was influenced by the effects of genotype. The yolk color value of the eggs in all interactive groups varied from 9.40 to 11.20. Our results were found to be much greater than the findings of Son et al.<sup>[52]</sup> for yolk color (from 7.50 to 8.40) in eggs of laying hens. The hen itself, the environment, correct husbandry practices, and good quality feed are very important to deliver an attractively pigmented yolk.

The haugh unit is the most widely used measure of the freshness of eggs and reflects the quality of eggs all over the World <sup>[11,53]</sup>. Albumen height, yolk height, HU, and yolk pH are good indicators of storage time, and it can be assumed that it is important to consider that eggs were fresh and of good quality <sup>[10,13]</sup>. In this study, the Haugh unit values were significantly affected by the genotype and length of the egg storage period. Commercial Nick Brown had significantly greater HU, while Atak-S, Sussex, and cross-breed had similar HU values. In agreement with the findings of Federn et al.<sup>[13]</sup>, Kralik et al.<sup>[49]</sup> and Petek and Abdourhamane [54] the HU values calculated in our study quickly decreased with the length of storage period. Average HU values of fresh eggs from all genotypes were found to be acceptable for the AA grade standard of table eggs at a score of 72 or above [55]. Eggs of Nick Brown and Sussex at 15 days of storage also met the standard value for HU. Son et al.<sup>[52]</sup> reported that HU varied from 74.9 to 77.9 in laying hens. Genotype had a significant effect on Haugh units, and commercial laying hens, in general, had a higher HU value than pure-breed or local layers. In another study, Rizzi [35] showed that the eggs of Hy-line white showed a greater HU value than the eggs of Hy-line Brown, local Robusta Maculata, and Ermellinata di Rovigo breeds. Kejela et al.<sup>[38]</sup> reported that the Haugh unit of the eggs of the native chickens was 74.91 and 82.55, while for Sasso chickens were 86.50 and 87.04, and Bovans brown were 94.60 and 86.29, respectively.

The external and internal egg quality characteristics of poultry eggs are affected by a variety of factors such as genotype, nutrition, housing conditions, animal health, stage of laying period, environmental condition, length of egg storage, and storage conditions. Besides these factors, some external quality parameters such as egg size, eggshell thickness, shape index, eggshell color, and thickness might have an effect on internal egg quality. In general, the free-range eggs had superior egg quality parameters compared to the eggs from colony cages <sup>[1,56]</sup>. If we will implement cross or pure-breed laying hens in commercial

egg production, it is necessary to identify these birds according to other production criteria such as egg weight and egg weight increase rate, shell strength, dry matter of egg albumen or yolk ratio. In our study, daily fresh eggs in all genotypes exhibit high-quality measurement according to their HU values, and eggs produced by Nick Brown and Sussex met an A-graded egg standard even until the 15 d of storage. Therefore, the laying hen genotype should be taken into account when determining the maximum storage time in room or refrigeration conditions. It should be best to consume the eggs as fresh as possible because the persistence of egg quality tends to decline due to the length of the egg storage period. The eggs of the commercial Nick Brown genotype seems slightly superior to those of other group eggs, while Sussex and Atak-S had similar external quality characteristics. Further studies, including production traits, economics, and the influence of consumer interest, are required for more profitable and sustainable egg production when choosing the laying hen genotype for non-cage systems, especially for free-range housing.

### **Declarations**

**Availability of Data and Materials:** Datasets used in this experiment are available from the corresponding author (MP) on request.

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Author Contributions: Conceptualization: MP, Data curation: MP, Formal analysis: MP, Funding acquisition: MP, Investigation: MP, Methodology: TK, Project administration: MP, Resources: MP, Software: TK, Supervision: MP, Validation: MP, Visualization: MP, Writing - original draft: MP, Writing-review and editing: MP

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