Original article

External and internal quality traits of eggs from different ornamental chicken breeds

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Abstract

This study analyzed the internal and external quality traits of eggs derived from hens of different breeds, including Silkie, Sultan, Cochin Bantam, Brahma and White Leghorn. The highest mean weight was noted for eggs originating from the White Leghorns breed, and the lowest was for eggs from the Cochin Bantams. Simultaneously, both a positive correlation between the egg weight and the percentage of albumen ($r = 0.876$) and a negative correlation between egg weight and the percentage of yolk ($r = -0.842$) were found. The eggshell composition varied significantly in mean phosphorus amount, whereas the calcium content did not differ significantly. Despite this, eggshell strength varied significantly between breeds. Regarding cholesterol and fatty acid levels, the highest amount of cholesterol was noted in the Cochin Bantam breed, and the lowest was in the White Leghorn, although Leghorn was the breed characterized by the highest saturated fatty acid levels, and Cochin Bantam was the lowest. Regarding the polyunsaturated fatty acids (which have been proven to positively influence the cardiovascular system), the highest levels were obtained by Leghorn eggs, and the lowest were obtained by Silkie eggs. In conclusion, the study indicates that ornamental chicken breeds are a source of high-quality products, which could be attractive to consumers, additionally supporting traditional farming and animal genetic resources.

Keywords: chicken breeds, egg quality, fatty acids, cholesterol

Introduction

Eggs, due to their high nutrient values, have been one of the most common and versatile food items since the prehistoric period (Song et al. 2000). Egg production in EU-27 countries in 2020 amounted to over 6.9 million tons. Poland is ranked sixth in terms of egg production among EU member states, with a production rate at the level of 9%, followed by countries such as France, Denmark and Italy (https://agriculture.ec. europa.eu/farming/animal-products/eggs_en).

Currently, the world poultry market is dominated by the commercial segment, focused on indoor environments and automated production systems and processes (Fraser 2008). For the egg industry the production economy has the greatest significance. According to

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Hrnčár et al. (2013), the major factor affecting the economy of production is eggshell quality, since cracked eggshells present higher losses for egg producers. Eggshell quality can be described as eggshell thickness and eggshell strength. The organization of eggshell microstructure is determined by genetic, physiological and external factors (Rodriguez-Navarro et al. 2007). Among external factors, calcium and phosphorus levels have a significant impact on eggshell quality (Arpášová et al. 2010).

On the other hand, for consumers, the crucial are nutritional and health benefits of eggs are crucial. The nutritional value of eggs is distinguished by the presence of essential lipids, vitamins, minerals and high-quality protein, with all of the essential amino acids (Nys and Sauveur 2004), and simultaneously, eggs are offering a moderate calorie source and great culinary potential (Réhault-Godbert et al. 2019). Eggs are defined as a functional food containing various bioactive compounds that can affect the proinflammatory and anti-inflammatory pathways. The egg can show immunomodulatory, anti-inflammatory, antioxidant, anticancer, and antihypertensive effects with its bioactive components (Sanlier and Ustun 2021). Also, the composition of fatty acids in egg lipids has especially been highly concerning for human health, such as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (Polat et al. 2013). For instance, an adequate intake of n6:n3 PUFAs ratio positively influences the prevention of cardiometabolic disease (Crowe-White et al. 2018). In addition, n-6 and n-3 PUFAs are metabolized to yield eicosanoids, which elicit physiologic effects influencing metabolic health, including inflammation and insulin sensitivity (Patterson et al. 2012). The fatty acid composition of poultry eggs may be affected, among others, by diet, the age of the hen or geographic location (Milinsk et al. 2003). However, eggs, even though a valuable source of many nutrients in the human diet, also contain components whose intake should be kept low, among which the most important is cholesterol. In line with recommendations of the American Heart Association (AHA), people should consume fewer than three whole eggs per week, which corresponds to a cholesterol consumption of no more than 300 mg/day (Krauss et al. 2001). This is crucial, since the relationship between dietary cholesterol and the risk of cardiovascular disease has been welldocumented. Moreover, Enjoji and Nakamuta (2010) underlined that excess cholesterol intake appears to be one of the main factors in the development of nonalcoholic fatty liver disease.

The industrialization and globalization of chicken production in the 20th century adversely affected the distribution of chicken genetic resources worldwide, practically limiting the breed composition to commercial stocks of broilers and egg-type, laying hens (https://www.iaea.org/resources/news-article/genetic-characterization-of-indigenous-chicken-breeds-in-search-for-unique-properties-of-immune-related-genes). Modern, large-scale, poultry product producing farms usually utilize cage housing systems, because of its ease of use, easy maintenance and ability to keep a larger number of hens in a smaller area. Even though the 1999/74/EC Council Directive clearly determines minimum conditions that must be provided for keeping laying hens, other research revealed that caged hens showed increased tonic immobility durations (which indicates fearfulness) and increased feather corticosterone concentration (an indicator of chronic stress) compared to hens that were housed in enriched pens (Campbell et al. 2022). Now, as ethical and environmental concerns regarding current poultry production systems arise, consumers are looking for alternatives (Escobedo del Bosque et al. 2021). Over the last decade, consumer willingness to pay for cage-free eggs has led to an increase in research in this area (Lusk 2019). Some research showed that people pay more attention to animal welfare issues than environmental concerns (Heng et al. 2013), and there is a demand for more natural, animal-friendly backyard egg production systems (Lemos Teixeira et al. 2018). Except for the high-productive lines and breeds of chicken, people often keep ornamental breeds, not only for aesthetic reasons and as a hobby, but also to produce a high number of eggs. Moreover, ornamental breeds provide a pool of potentially useful genetic resources for commercial layers (Ajayi 2010, Lordelo et al. 2020). According to Malomane et al. (2019) ornamental breeds, as highly diverse breeds, are maintained for the sustainability and flexibility of future chicken breeding. They should be perceived as valuable not only due to their unique appearance, but also for their egg production. Moreover, ornamental breeds are well characterized in terms of color, feathers, and morphological features (Bernacki and Kaszyński 2013) with low awareness about quality of eggs produced by them. Furthermore, the majority of studies focused on the quality of eggs derived from commercial breeds and the impact of diet fed on their physical characteristics, fatty acids and sensory properties (Drażbo et al. 2014).

The aim of this study was to determine the quality of eggs obtained from different ornamental chicken breeds. The five most commonly reared ornamental breeds, such as White Leghorn, Brahma, Cochin Bantam, Sultan, and Silkie, were selected for the study. All chosen breeds were standardized and Sultan was classified as Miscellaneous breed, White Leghorn

Table 1. Nutritional value of layers feed.

Ingredients	% composition
Total protein	15.5
Crude fat	5.40
Crude ash	4.00
Crude fibre	13.40
Lysine	0.74
Methionine	0.37
Calcium	4.00
Phosphorus (non phytin)	0.42

as Mediterranean breed, and Brahma, Cochin Bantam and Silkie as Asiatic breed (Allonby et al. 2018). Because ornamental breeds lay well, it seems crucial to characterize the properties of eggs derived from them. It has been well described that both genetic and environmental factors influence the quantity and quality of eggs (Goto and Tsudzuki 2017, Wilson 2017). Therefore, for better understanding of the impact of the genetic factors on traits of eggs, it is important to perform studies on different breeds reared under the same conditions and eating the same feed. In this study we aimed to reveal breed effect on external and internal traits of eggs, and to estimate the correlations between egg traits and fatty acid profile using 5 ornamental chicken breeds.

Materials and Methods

Egg sampling

This study was run in accordance with Directive no. 2010/63/EU and did not require the approval of the Local Ethics Committee based on the regulation of the Ethic Committee of November 2019 (resolution no. 174/2019). A total of 25 laying hens – 5 from each of the breeds: Leghorn, Brahma, Silkie, Cochin Bantam and Sultan, all at the age of 46 weeks (hatched in May 2020) were reared in a non-commercial farm located in middle-eastern Poland, in Mazovian region. The average hen body weight was approximately 2.2 kg for Leghorn, 3.7 kg for Brahma, 0.8 kg for Cochin Bantam, 1.6 kg for Sultan and 1.4 kg for Silkie. The hens were divided into five groups, one group per breed. Each group was in separate chicken coops with a bed of wood shavings and its own run. Each section was 4 x 2 m with a stocking density of 1.6 m^2 per 1 hen. Inside each chicken coop there was a straw-bedded nest. The birds were reared in the same environmental conditions and fed with the same feed ad libitum. Laying hens were fed mixtures whose nutritional value are given in Table 1. The metabolizable energy of their diet was 2800 kcal per 1 kg of feed. Altogether,

50 eggs obtained from different chicken breeds were tested, with 10 eggs from each breed being collected daily on different occasions and analyzed within 24 hours post-laying. The experiment was conducted over a 2-month period (April and May 2021).

Physical characteristics

Egg quality criteria were determined in 50 eggs, ten eggs per breed. The eggs were individually weighed on a Digital Analytical Balance (Radwag XA 110.4Y.A plus, d=0.01 mg). A Digital Vernier Caliper (General Tools & Instruments, USA) with a resolution of 0.01 mm was used to measure the width and length of the eggs. The shape index (SI) was calculated based on the ratio of egg width to egg length. Subsequently, eggshell strength was measured using an egg force reader (Orka Food Technology). The eggs were placed vertically, with the blunt end up, between two flat steel plates and compressed at a speed of 1 mm/min. The results were shown in kgf (kilogram-force) after each egg broke. The eggs were broken carefully, and the content (both yolk and albumen) was smoothly deposited on a flat glass plate. The height of the yolk was measured with a micrometer screw (d=0.01 mm), and the yolk diameter was measured with a Digital Vernier Caliper (General Tools & Instruments, USA), d=0.01 mm. The yolk index was calculated by dividing the yolk height by the yolk diameter (Funk 1948). Yolk color was evaluated visually with a Roche yolk color fan. A Digital Haugh Tester (Orka Food Technology) was used to measure albumen height. Three measurements were carried out at different points of the thick albumen at a 7 mm distance from the egg yolk and given as an average.

Using albumen height and egg weight, Haugh units (HU) were calculated based on the following formula (Haugh 1937):

$$HU = 100 * \log_{10}(H - 1.7 * W^{0.37} + 7.6),$$

where: H – albumen height (mm),

      W – egg weight (g).

To determine the yolk weight, the yolk was carefully separated from the albumen using an egg separator

and gently rolled on towel paper to remove the remaining albumen and was then placed on a Lab Digital Balance (Radwag, Poland).

Every eggshell was driedout at room temperature for 72 hours. After drying, each eggshell was weighed using a Lab Digital Balance (Radwag, Poland). Subsequently, the egg membranes were removed, and the eggshell thickness was measured using a thickness gauge (Mitutoyo, Japan), d=0.01 in three zones (blunt end, sharp end and the equator) and the mean of the three measurements was calculated. Albumen weight was calculated by subtracting the yolk and eggshell weight from the whole egg weight.

Chemical analysis

The level of fatty acids and cholesterol was measured in 40 egg yolks, using eight yolks from each group.

Fatty acid profile

Concentrations of FA (fatty acids) were determined in fresh eggs using the Folch method (Haugh 1937). Egg yolks (c. 5-6 g) were dissolved and homogenized in a chloroform-methanol mixture (2:1), and then filtrated. The extract obtained was rinsed with HPLC-MS grade water and left for 12 hours to separate the phases. The non-polar phase with yolk fat was separated and evaporated using a Rotary Evaporator (Heidolph Instruments, Germany). Following this, 50-60 mg of the remaining fat was transferred to 2 cm^3 ampoules, 1500 µL of methanol-chloroform-sulphuric acid (100:100:1 v/v) mixture was added, and the ampoules were closed by melting their tops with an open flame. Closed vials were placed in a dryer at 90°C and were heated for 120 min. After solvent evaporation and ampoule cooling, 0.5-1 mL of hexane was added to each sample and thoroughly mixed. The resulting fatty acid methyl esters (FAMEs) were analyzed using a Unicam PU 4600 gas chromatograph with flame--ionization detection. The fatty acid methyl ester was injected at 290°C, separated in a capillary column (30 m, 0.40 mm, 0.25 µm; Supelcowax 10) and detected via flame-ionization at 300°C; carrier gas: argon; flow rate: 50 mL/min. The peaks of fatty acids were identified by comparing their relative retention times with those of individual FAME reference standards (Supelco, Sigma Aldrich group) diluted in hexane (1:1,1:2,1:3,1:4 v/v).

Cholesterol levels

Cholesterol was separated from the fat, as described previously by Folch et al. (1957), after saponification with KOH and extraction with ethyl ether, using the modified method of the International Dairy Federation (1992). The samples were subjected to an HPLC analysis in a Merck UV-VIS chromatograph (Germany) according to the Nogueir and Bragagnolo method (2002), under the following conditions: column: LiChzocard C$_{18}$ (125 x 4 mm), wavelength: 210 nm. The mobile phase was: methanol:acetonitrile (95:5 v/v) at a flow rate of 1 mL/min. Internal standard: dotriacontane C$_{32}$H$_{66}$ (Sigma, St. Louis, MO). Cholesterol content was calculated and expressed as milligrams per gram of yolk fat.

Ca and P content in eggshells

The eggshells collected from all breeds were washed with water, dried in air and powdered. 100 mg of each sample was then weighed and placed in a digestion PTFE vessel, 8 mL of 65% nitric acid (Suprapur; Merck, Darmstad, Germany) was then added, and the samples were placed in Titan MPS microwave sample preparation system (Perkin Elmer, Waltham, MA). The sample digestion was performed in accordance with the following program. The 1st step was performed at 160°C, ramp was 10, hold was 10 and the P value was 80%. The 2nd step was performed at 190°C with the ramp at 5, hold 20 and the P value was 90%. The 3rd step took place at 50°C with the ramp at 1 and the hold was 10. The P value was 0%. In all 3 steps the pressure was 30 bar. After digestion, the samples were quantitatively transferred into 50 mL flasks and filled up to the mark with 1% nitric acid solution. Because of the expected high concentrations of calcium, all the previously prepared samples were diluted in a 1:250 1% nitric acid solution to enable an analysis of the Ca concentration.

An inductively coupled plasma-optical emission spectrometer (Optical Emission Spectrometer Avio TM 200, Perkin Elmer) was used for the measurements of the following elements: Ca and P. The setup parameters of the spectrometer were: Plasma gas flow 10 L/min, auxiliary gas flow 0.2 L/min, gas flow through atomizer 0.7 L/min, plasma power 1500W, plasma observation height 15 mm, sample flow 1 mL/min and the delay time 30 sec. The wavelength was set as 317,938 nm for Ca, 213,622 nm for P and 371,029 nm for Yttrium.

Calibration and Accuracy of the Method: Calibration of the spectrometer for each element was performed using aqueous calibration standards. Calibration solutions were prepared from a 1000 mg∕L stock solution (Merck) by dilution in 1% (v/v) HNO$_3$. For each element, five standard solutions of different concentrations were prepared. The ranges of calibration curves were as follows: 0-50 mg∕L for P; 0-100 mg∕L for Ca. Yttrium was chosen as the internal standard.

Table 2. Internal and external quality traits of eggs.

	Leghorn	Brahma	Cochin b.	Sultan	Silkie	P value
Egg weight [g]	63.63[a]±3.98	55.85[b]±2.64	30.29[c]±1.38	44.88[c]±1.57	41.15[d]±0.97	0.000
Shape index (SI)	75.05[b]±1.79	73.25[b]±2.29	78.58[a]±1.31	77.77[a]±1.91	77.95[a]±0.90	0.000
Yolk index (YI)	42.92[b]±3.37	44.64[ab]±2.00	46.60[a]±1.77	38.86[c]±2.69	45.25[ab]±2.61	0.000
Yolk color	9.7[b]±1.49	11.3[a]±1.27	10.0[b]±0.89	9.1[b]±1.76	10.1[ab]±0.94	0.016
Albumen height [mm]	8.07[a]±1.49	6.61[b]±0.71	5.38[c]±0.50	5.59[c]±0.85	5.58[c]±0.75	0.000
Haugh unit (HU)	88.10[a]±9.27	82.28[ab]±4.28	84.28[ab]±2.65	79.06[b]±6.57	80.73[ab]±4.90	0.025
Yolk content [%]	24.05[d]±1.70	29.35[c]±2.10	34.57[a]±0.89	31.50[b]±1.45	30.90[b]±0.63	0.000
Albumen content [%]	66.75[a]±1.66	61.60[b]±1.97	55.68[d]±1.07	59.40[c]±1.59	59.11[c]±0.54	0.000
Eggshell content [%]	9.20[bc]±0.32	9.05[c]±0.45	9.75[ab]±0.42	9.10[c]±0.42	9.98[a]±0.46	0.000
Eggshell thickness [mm]	0.33[a]±0.02	0.31[ab]±0.02	0.27[c]±0.02	0.29[b]±0.01	0.32[a]±0.03	0.000
Eggshell strength [kgf]	3.34[bc]±0.69	4.04[ab]±0.43	2.98[c]±0.41	3.195[c]±0.52	4.34[a]±0.54	0.000
Ca in eggshell [%]	35.63±0.98	35.63±1.82	34.49±1.74	35.32±2.12	35.16±1.90	0.638
P in eggshell [%]	0.13±0.05	0.161±0.04	0.15±0.04	0.164±0.03	0.20±0.06	0.120

a, b, c, d, e – Means within a row with different superscripts differ significantly (p<0.05)

Table 3. Correlations of internal egg quality characteristics.

	Shape index	Yolk index	Haugh unit	Yolk content	Albumen content	Cholesterol content
Egg weight	- 0.656	- 0.239	0.177	- 0.842*	0.876*	- 0.074
Shape index		0.011	- 0.183	0.476	- 0.518*	- 0.269
Yolk index			0.408*	0.103	- 0.145	0.296
Haugh unit				- 0.251	0.240	0.134
Yolk content					- 0.990*	0.228
Albumen content						- 0.229

* p<0.05

Statistical analysis

A statistical analysis was conducted using Statistica version 13.3 software (Statsoft, Poland). The data were expressed as mean (± standard deviation (SD). Shapiro-Wilk's test was used to analyze the normal distribution of values. To identify the differences between each treatment, a one-way ANOVA was performed, followed by the Tukey test. For abnormally distributed data, the Kruskal-Wallis test was performed, followed by a double comparison (the Mann-Whitney test). The significance was determined at p<0.05. Pearson's correlation coefficients between the different parameters were calculated.

Results

Physical characteristics

There were statistically significant differences between breeds for the majority of physical characteristics tested (Table 2). The average weight of eggs varied from the lowest in the Cochin Bantam breed to the highest in the Leghorn breed (p<0.05).

Moreover, a positive correlation between the egg weight and percentage of albumen and a negative correlation between egg weight and percentage of yolk were found (Table 3).

The Cochin Bantam hens laid the lightest eggs with the lowest percentage of albumen and the highest percentage of yolk, while Leghorn hens laid the heaviest eggs with the highest percentage of albumen and the lowest percentage of yolk. Additionally, there were significant differences in Haugh unit (HU) and yolk index (YI) between eggs derived from different breeds.

Table 4. Correlations of external egg quality characteristics.

	Shape index	Eggshell content	Eggshell thickness	Eggshell strength	Calcium content	Phosphorus content
Egg weight	- 0.656*	- 0.502*	0.583*	0.157	0.224	- 0.157
Shape index		0.445*	- 0.299*	- 0.165	- 0.083	0.152
Eggshell content			0.169	0.229	- 0.187	0.024
Eggshell thickness				0.589*	0.154	- 0.060
Eggshell strength					0.090	0.087
Calcium content						0.284*

* $p < 0.05$

The highest mean value of YI was observed in eggs derived from Cochin Bantam hens and the lowest was observed in eggs from the Sultan breed ($p < 0.05$). HU values (describing albumen quality) were noted as the lowest in the Sultan hens and the highest in the Leghorn group ($p < 0.05$).

In relation to yolk color, the most hued egg yolks were collected from the Brahma group and the palest were from the Sultan breed.

The calculated egg shape index (SI) also differed significantly between breeds ($p < 0.05$). Moreover, a significant negative correlation between egg shape index and egg weight was reported (Table 4). Similarly, there were significant differences in the shell content of eggs derived from different breeds (results varied from the lowest value in Brahma to the highest in Silkie hens). The breed had an impact on the shell thickness, and the lowest value observed was obtained from Cochin Bantam hens, while the highest was in the Leghorn breed ($p < 0.05$). Inherent to eggshell thickness are the eggshell strength and the mineral content of Ca and P. The strength of eggshells varied from the lowest in eggs derived from the Silkie breed to the highest in the Cochin Bantam breed. There were no statistically significant differences in the level of calcium between eggs derived from different breeds. In contrast to calcium concentration, the phosphorus amount in eggshells differed significantly between breeds. The highest result was noted in the Silkie breed and the lowest in the Leghorn breed.

Fatty acid profile

The composition and content of fatty acids are given in Table 5. The percentage of saturated fatty acids (SFAs) in eggs was at a similar level in the majority of tested breeds with significantly lower content in the Cochin Bantam breed. The most abundant was palmitic acid C 16:0 (the lowest result in the Cochin Bantam group and the highest in the Brahma group), regardless of the origin of eggs, while the least was pentadecanoic acid C 15:0 (the lowest value in Brahma eggs and the highest in Sultan eggs).

In relation to the content of monounsaturated fatty acids (MUFAs), significantly lower values were observed in eggs derived from Leghorn and Sultan breeds compared to eggs derived from the remaining breeds ($p < 0.05$). Irrespective of the breed, oleic acid (C 18:1 cis-9) was the most abundant MUFA in all tested eggs ranging from the lowest result in Leghorn to the highest in Silkie, while myristoleic acid (C 14:1) was at the lowest level (ranging from the lowest value obtained in the Sultan breed to the highest in the Brahma breed).

Similarly, there were differences noted in PUFAs content and their composition in egg yolk, depending on the breed. The largest amount of PUFAs was noted in eggs originating from the Leghorn breed and the lowest was in the Brahma breed. Across all the breeds, the most abundant PUFA in eggs was linoleic acid (C 18:2), ranging from the lowest level in the Brahma group to the highest in the Sultan group, while the least abundant was dihomo-γ-linolenic acid (C 20:3 n6) ranging from the lowest value in the Sultan breed to the highest in the Leghorn breed.

The impact of breed on n6/n3 fatty acid ratios in the yolk of eggs was also noted ($p < 0.05$). The lowest ratio was observed in eggs laid by the Leghorn breed and the highest was noted in the Sultan breed.

Cholesterol levels

The mean yolk cholesterol content in eggs laid by hens of different breeds is presented in Table 5. The cholesterol levels ranged from the lowest result in Leghorn eggs to the highest in Cochin Bantam eggs ($p < 0.05$). Moreover, there was a weak correlation between the percentage of yolk in the egg and cholesterol content (Table 3).

Discussion

The influence of hen breed on egg quality has been widely described and confirmed by many authors (Lordelo et al. 2020, Nolte et al. 2021), and this correlation has a significant impact taking into consideration

Table 5. Fatty acid composition and cholesterol levels.

	Group					P value
	Leghorn	Brahma	Cochin	Sultan	Silkie	
Total SFA, including:	35.94[a]±1.12	35.93[a]±1.50	32.96[b]±0.35	35.74[a]±0.66	34.7[a]±0.91	0.000
C 14:0	0.38±0.08	0.38±0.04	0.34±0.04	0.35±0.01	0.37±0.01	0.188
C 15:0	0.09±0.01	0.05±0.01	0.06±0.01	0.15±0.00	0.07±0.01	0.276
C 16:0	25.99[ab]±0.96	26.7[a]±1.44	24.67[c]±0.35	25.4[bc]±0.59	25.5[abc]±0.64	0.001
C 17:0	0.27[a]±0.03	0.15[c]±0.03	0.17[bc]±0.02	0.21[b]±0.01	0.19[b]±0.03	0.000
C 18:0	9.01[ab]±0.41	8.45[bc]±0.56	7.51[d]±0.29	9.41[a]±0.18	8.39[c]±0.43	0.000
C 22:0	0.19±0.07	0.2±0.02	0.21±0.07	0.23±0.03	0.18±0.04	0.530
Total MUFA, including:	44.85[b]±2.71	49.01[a]±2.51	48.38[a]±1.00	45.51[b]±2.00	49.37[a]±0.91	0.000
C 14:1	0.06[b]±0.02	0.09[a]±0.03	0.07[ab]±0.01	0.05[b]±0.01	0.05[b]±0.01	0.000
C 16:1	2.52[c]±0.57	3.7[a]±0.66	3.38[ab]±0.31	2.4[c]±0.24	2.95[bc]±0.24	0.000
C 17:1	0.17[a]±0.02	0.13[b]±0.03	0.16[a]±0.02	0.15[ab]±0.01	0.16[a]±0.02	0.002
C 18:1 cis-9	40.07[b]±2.69	42.37[ab]±2.70	41.73[ab]±0.73	41.01[ab]±1.77	43.57[a]±0.98	0.012
C 18:1 cis-11	1.76[c]±0.12	2.4[b]±0.27	2.72[a]±0.17	1.63[c]±0.08	2.32[b]±0.18	0.000
C 20:1	0.27±0.05	0.32±0.04	0.31±0.03	0.27±0.02	0.31±0.04	0.560
Total PUFA, including:	19.21[a]±2.25	15.06[b]±1.64	18.66[a]±0.95	18.75[a]±2.50	15.94[b]±1.54	0.000
C 18:2	14.79[a]±1.98	11.41[c]±1.57	14.15[ab]±0.93	14.94[a]±2.33	12.3[bc]±1.45	0.000
C 18:3	0.66[a]±0.16	0.32[b]±0.06	0.34[b]±0.12	0.43[b]±0.10	0.43[b]±0.12	0.000
C18:3 γ-lin	0.18[a]±0.02	0.14[bc]±0.04	0.14[bc]±0.01	0.17[ab]±0.02	0.12[c]±0.02	0.000
C 20:2	0.16[ab]±0.04	0.14[b]±0.03	0.19[a]±0.02	0.14[b]±0.02	0.13[b]±0.02	0.000
C 20:3 (n-6)	0.16[a]±0.02	0.13[b]±0.02	0.18[a]±0.01	0.11[b]±0.01	0.12[b]±0.01	0.000
C 20:4 (n-6)	1.93[bc]±0.21	2.11[b]±0.17	2.49[a]±0.13	1.83[c]±0.07	1.91[bc]±0.26	0.000
C 22:5 (n-6)	0.40[b]±0.08	0.31[bc]±0.09	0.53[a]±0.09	0.55[a]±0.08	0.28[c]±0.06	0.000
C 22:6 (n-3)	0.93[a]±0.11	0.49[c]±0.12	0.64[bc]±0.04	0.57[bc]±0.05	0.66[b]±0.18	0.000
n3	1.58[a]±0.23	0.82[c]±0.1	0.98[bc]±0.15	1.00[bc]±0.15	1.08[b]±0.22	0.000
n6	15.54[a]±2.1	12.00[c]±1.57	15.01[ab]±0.9	15.80[a]±2.3	12.82[bc]±1.43	0.000
n6/n3	10.03[c]±2.1	14.96[ab]±2.87	15.6[ab]±2.1	15.77[a]±1.02	12.30[bc]±3.03	0.000
cholesterol (mg/g of yolk fat)	31.96[b]±7.25	44.44[ab]±7.63	52.20[a]±14.52	35.3[b]±7.93	38.69[b]±11.43	0.000

a, b, c, d – Means within a row with different superscripts differ significantly (p<0.05)

the preferences of consumers. According to Arthur and O'Sullivan (2005), worldwide, egg customers have much in common, generally preferring eggs which have a sound shell, uniform shell color, freedom from obvious blood and meat spots and a reasonably upright egg white. Moreover, Drabik et al. (2021) emphasised that egg quality is also determined by factors such as the age of the birds, housing system, and feeding regime. The results presented by many authors usually refer to eggs obtained from commercial hybrid hens, although there is much less data on the quality of eggs obtained from ornamental chicken breeds.

Egg weight is a crucial criterion that affects the retail value of eggs. In the current study, significant differences in egg weight were found between tested breeds. Interestingly, this physical trait is not correlated with the body weight of the birds. The heaviest eggs were obtained from the Leghorn hens, and the lightest

eggs were obtained from Cochin Bantam hens, while the hen body weights varied from the lowest in Cochin Bantam to the highest in Brahma. Moreover, many authors noted that egg shape significantly affects the shape index (She et al. 2009, Aygun and Yetişir 2010, Duman et al. 2016). As mentioned by Shaker et al. (2017), shape index (SI) may be considered as a good indicator to characterize species as well as egg quality, and the authors suggested that shape index is affected by genetic and environmental factors. The eggs are classified with respect to shape index as sharp eggs (SI<72), oval eggs (SI=72-76) and round eggs (SI>76) (Duman et al. 2016). Interestingly, the most desired are eggs with SI ranging from 72 – 76, because this is directly correlated with the mechanical properties of eggs. Altuntaş and Şekeroğlu (2008) also showed that these eggs are the most resistant to compression. In the current study, two out of five breeds, Leghorn

and Brahma, were laying eggs characterized as "oval". For eggs obtained from the remaining breeds, their SI values were slightly higher than 76, and these eggs were described as "round". In a study conducted by Kaszyński and Bernacki (2014), the SI of eggs laid by Silkie hens increased during the laying period and was from 71.56 in the early egg production period up to 77.63 at the end of the laying period, which resulted in a mean value of 74.78. It is worth noting that since that study was conducted at the end of the laying period, these findings were close to the current results. Similarly, research carried out by Hrnčár et al. (2015) revealed that the SI of Brahma eggs was 76.24, which was slightly higher than the current result; however, the observed values did not differ significantly. According to Liswaniso et al. (2020), the correlation between the egg weight and egg shape index is triggered by the presence of the heavier part of the egg (egg white) in its wider part. These findings are in accordance with the current studies, in which, as the egg weight increases, the weight of the albumen increases, resulting in a decrease in the proportion of yolk to albumen. Studies performed by Ianni et al. (2021), also showed that the heaviest eggs collected from commercial hybrid hens were characterized by a lower proportion of yolk (24.5%) compared to eggs obtained from Nera Atriana hens, which were lighter, but had a higher yolk amount (29.09%). The current authors found that the yolk to albumen ratio ranged from 0.36 in Leghorn to 0.62 in Cochin Bantam, and these findings are in accordance with studies performed by Moula et al. (2009) showing that the yolk/albumen ratio is negatively correlated with egg weight.

It has been commonly assumed that the main indicators to measure the quality of eggs are the Haugh unit and the yolk index (Shi et al. 2009). The yolk index is a utility that describes yolk flattening, and it gives information about egg freshness. The yolk index decreases when the vitelline membrane becomes weaker and allows water to migrate from the albumin (Jones and Musgrove 2005). This phenomenon is intensified over the storage period, but the higher the initial value is, the slower it progresses. Eggs with a YI value higher than 38 are classified as "extra fresh". Values between 38-28 are "fresh", and eggs with YI lower than 28 are "regular". In the current study, since the YI varied from 38.86 in the Sultan breed to 46.60 in the Cochin Bantam, all eggs were classified as "extra fresh".

The Haugh unit, which was introduced by Haugh in 1937, is an index that adjusts the height of the thick inner albumen according to the weight of the egg and relates well to the egg quality (Rafea 2019, Hisasaga et al. 2020). The higher the HU value, the bet-

ter the albumen quality of the eggs (Stadelman 1995). Generally, an HU greater than 72 indicates an AA grading for the egg, and AA is the highest quality egg, followed by A and B (Jones 2012). In the current study, all eggs derived from the different breeds were labeled as AA grade. Similarly, in a survey of the quality of brown eggs derived from five brands in the USA, most brands were AA quality (Hisasaga et al. 2020). Studies performed by Jones and Musgrove (2005) indicated that extended cold storage led to decreases in egg weight, albumen height and HU.

The yolk color has a relevant influence on the perception of egg quality by consumers. The current results showed that breed had a significant impact on yolk color. It is generally considered that the differences in yolk color are influenced by the feed additive; Grashorn (2016), and Hammershøj and Johansen (2016) stated that the grasses and herbs consumed by free-range hens have an impact on yolk color intensity. However, Hanusova et al. (2015) and Krawczyk (2017) revealed the prevalence of diversity in yolk color between hens of different genotypes. These authors also noted that individual birds absorb xanthophyll pigments to a large extent.

Eggshell quality traits play an important role because only eggs with an intact shell are considered for hatching or as table eggs (Ketta and Tumova 2017). Therefore, eggshell strength and other eggshell characteristics, such as eggshell thickness, are the major indirect parameters for the evaluation of eggshell quality (Yan et al. 2014). In the current study, it was found that breed significantly impacts eggshell strength, and the eggshell strength was increased with eggshells becoming thicker. However, the highest and the lowest breaking strength was noted in eggs laid by Silkie and Cochin Bantam, while Cochin Bantam and Leghorn had the thinnest and thickest eggshells, respectively. According to Bain (2005) although thickness is the main factor contributing to the mechanical strength of an eggshell, thicker eggshells do not guarantee stiffer or stronger eggs. Considering the findings noted by Stadelman (1995) that an eggshell thickness of at least 0.33 mm is necessary for the eggs to have at least a 50% chance to withstand normal handling conditions without breakage, among the tested breeds, only Leghorn hens laid eggs with appropriate shell thickness.

The current study found a very weak correlation between the eggshell strength and the amount of P and Ca. Calcium is the main mineral component of the eggshell, with the most common crystalline form of calcium being calcium carbonate ($CaCO_3$) with 93.6%, followed by calcium triphosphate (0.8%) and magnesium carbonate (Neunzehn et al. 2015). Therefore, the critical component for shell strength and integrity

is appropriate calcium and phosphorus metabolism and utilization. However, older hens may have a reduced ability to absorb Ca from the diet, requiring constant supplementation of this nutrient in the feed. In addition, as the hen ages and the eggs become larger, a similar amount of calcium must be spread over a larger area, thus reducing the thickness and, consequently, the strength of the shell (Świątkiewicz et al. 2018). Rodríguez-Navarro et al. (2002) reported that eggshells from aged hens had lower strength against breakage and showed greater variability in their structural properties, such as thickness, grain morphology and crystallographic texture. According to Park and Sohn (2018), due to the physiological characteristics of the hen and the physiochemical properties of the eggshell, it is posited that hen aging reduces the strength of the eggshell and degrades eggshell quality.

The fatty acid composition of the eggs is highly variable, probably reflecting differences in the diets of hens (Fraeye et al. 2012). This statement was confirmed by Kosewski et al. (2021), who found significant differences in the percentage of saturated fatty acids in egg yolks depending on the hen breeding method. However, in the current study, differences in the fatty acid compositions of yolk derived from different breeds were noted even though they were fed the same feed. Polat et al. (2013) also noted the differences in concentrations of fatty acids among eggs derived from poultry species kept in their natural environment. Additionally, the differences in the percentage of fatty acids between breeds did not affect their composition – in all breeds, palmitic acid was the main SFA, oleic acid was the main MUFA, and linoleic acid was the main PUFA. These acids were also noted as prevailing in yolks of chicken eggs derived from organic, free-range and cage farming (Kosewski et al. 2021) or in eggs derived from chicken, goose, duck, turkey, peacock and pheasant (Polat et al. 2013).

One of the most important and quality-determining compounds of eggs are omega-3 (n-3) and omega-6 (n-6) PUFAs, which must be provided by food because they cannot be synthesized in humans (Kaur et al. 2014, Mariamenatu and Abdu 2021). However, the n6:n3 ratio is crucial, as it is an important determinant of adequate fatty acid intake as well as preventing the occurrence of diseases (Hamady 2013, Neijat et al. 2016). Conventional hen diets result in eggs with a n6:n3 ratio of about 13:1 (Hamidu et al. 2022), which corresponds with the current findings. However, the currently recommended ratio of omega-6 to omega-3 intake is 4 to 1, as this proportion has an impact on cardiometabolic health and may reduce the risk of thrombosis, vascular wall inflammation and myocardial arrhythmia (Crowe-White 2018). Taking into consi-

deration cardiovascular disease, people should limit their intake of cholesterol, and eggs, especially the yolk, are a major source of dietary cholesterol. A large egg, approx. 50 g, contains approximately 186 mg of cholesterol (Hamidu et al. 2022). In the current study, the cholesterol levels were the lowest in Leghorn eggs and the highest in Cochin Bantam eggs. Cholesterol levels depend on many factors, including the origin of the hens, their age, the housing system and the type of feed consumed. According to Basmacioglu and Ergul (2005), egg cholesterol levels have been shown to vary with the species breed or strain of the bird. Rizzi and Chiericato (2010) add that a higher concentration of cholesterol in the yolk is typical for eggs from native breeds in comparison with commercial hybrids, which is caused by the lower laying intensity of native breeds. Zemková et al. (2007) showed that the concentration of cholesterol in the yolk is influenced by both the housing system and the age of the laying hens.

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