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Physical and biochemical characteristics of Japanese quail (*Coturnix japonica*) eggs based on shell color

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Ten percent of the world's total edible eggs come from Japanese quail. The popularity of quail eggs is attributed to their high yolk content and hypoallergenic properties. This study aimed to analyze the physical characteristics and selected biochemical parameters of Japanese quail eggs based on shell color (dotted - D group, spotted - S group, and hazy - H group). The heaviest and most elongated eggs were found in the group H. The yolk index was significantly higher in dotted eggs, which also exhibited the highest lysozyme content and activity. The thickest shell ($p \leq 0.05$) with the lowest elasticity was observed in hazy eggs. However, no differences were found between eggshell colors concerning their breaking strength. The highest Ca content in the shell was found in the spotted group (S), while hazy eggs exhibited the lowest ($p \leq 0.05$) Ca and Mg content, despite having the thickest shells. Hazy eggs were also characterized by the highest crude protein content and the smallest proportion of crude fat and ash. The dotted eggs had the highest ($p \leq 0.05$) proportions of C16:1 and C18:2 fatty acids and a higher PUFA content ($p \leq 0.05$). Nutritionally, the dotted eggs performed best in terms of fatty acid profile and were also characterized by higher Mg, Ca, Cu, Mn, K, and Na content compared to the other groups. In summary, dotted eggs may be more appealing to consumers due to their advantageous physical and biochemical characteristics.

KEY WORDS: Japanese quail / eggshell color / egg physical and biochemical feature

The global population of Japanese quail constitutes almost 12% of all poultry species, and 10% of the world's total table eggs come from these birds. Quail egg production is around 1.3 million tons annually, with most produced in Asia. China is the world's largest producer (184 billion eggs per year), followed by Thailand and Indonesia. In the European Union, France, Italy, and Spain are the top three producers, but the number of eggs produced in Europe is small compared to Asia [Bertechini and Oviedo-Rondon 2023].

While chicken eggs dominate the market, the significant contribution of Japanese quail eggs should not be overlooked. The popularity of these eggs is due in part to their high yolk content (often over 30%) and their reputed hypoallergenic properties [Benichou *et al.* 2014, Hrnčár *et al.* 2014]. Consumer interest has led to the market introduction of quail eggs enriched with omega-3, omega-6 fatty acids, squalene, and lycopene.

As with chickens (*Gallus domesticus*) or common pheasants (*Phasianus colchicus*), the shell color of Japanese quail eggs can vary significantly, with occurrences of bright, dotted, spotted, dark, white, and blue eggs [Taha 2011, Farghly *et al.* 2015, Lan *et al.* 2021]. Shell color is genetically determined, and in domestic chickens, it is associated with a specific genotype – breed or hybrid [Drabik *et al.* 2021]. However, specific chicken populations also exhibit variability in eggshell color [Rashed *et al.* 2023]. Similar variability has been observed in pheasants and quails. Studies on pheasants have linked eggshell color to external and internal quality traits [Kirikçi *et al.* 2005, Krystianiak *et al.* 2005]. For example, blue-shelled pheasant eggs are of lower quality, particularly in terms of shell strength [Kożuszek *et al.* 2009b].

Reproductive outcomes can also be linked to external and internal egg quality traits. Blue pheasant eggs, for example, have poorer hatchability, and excluding them from incubation is economically recommended [Kożuszek *et al.* 2009a]. Interestingly, no such relationship has been confirmed for blue-shelled Japanese quail eggs [Taha 2011]. Shell color may also be indirectly related to their biological value.

Previous studies have focused on the physical and biochemical traits of quail eggs, but they typically do not distinguish the eggs based on shell color [Gomathi *et al.* 2014, Tolik *et al.* 2014]. On the other hand, studies in which the experimental factor was the shell color of Japanese quail eggs are limited to the physical traits of the eggs [Alasahan *et al.* 2015], the course of embryogenesis, hatchability parameters [Taha 2011, Farghly *et al.* 2015, Ismael *et al.* 2024], and production parameters, such as slaughter traits [Alasahan *et al.* 2016]. No research results have been found regarding the relationship between shell color and the biochemical properties of the yolk and egg albumen, which would undoubtedly enrich and complement the existing knowledge about Japanese quail eggs. These results could be precious to the nutritional value essential to consumers.

Therefore, this study aimed to analyze the physical characteristics and selected biochemical traits of Japanese quail eggs based on shell color.

Material and methods

The experimental material consisted of Japanese quail eggs laid by 16-week-old females. The birds were housed in cages typical of egg production systems. Environmental conditions including cage equipment and stock density complied with the regulation of the Minister of Agriculture and Rural Development dated 19 September 2019 on minimum conditions for the maintenance of farm animal species other than those for which protection standards are specified in the regulations of the European Union (Journal of Laws of 2019, item 1966). The birds were fed a complete diet containing per 1 kg: 2600 kcal/kg metabolizable energy, 21.0% crude protein, 3.80% crude fiber, 11.00% crude ash, 2.95% calcium, and 0.6% phosphorus.

A total of 480 eggs were collected on the same day, examined for defects (these eggs were excluded from the examination), and classified into color groups:

– dotted – white to cream background with small spots (Fig. 1).
– spotted – cream to light brown background with average to large irregular spots (Fig. 2).
– hazy – shells with small to average spots and a matte, hazy-tinged surface (Fig. 3).


Fig. 1. Dotted eggs.


Fig. 2. Spotted eggs.


Fig. 3. Hazy eggs.

Egg physical traits

The number of analyzed eggs varied (dotted – 32; spotted – 35 and hazy – 30) due to damage during storage or measurement was shown in each table. Eggs were analyzed after 2 days of storage, and the following traits were recorded:

– egg weight (g) using a WPS 360C type balance (0.01 g accuracy)

– the egg shape was determined based on length and width measurements (using slide calipers with 0.01 mm accuracy). The egg shape index (%) was calculated according to the formula:

Egg shape index = egg width (mm) × 100/egg length (mm),

– yolk, albumen, and eggshell proportions (%) to initial egg weight as 100%,

– yolk index (%) was calculated according to the formula:

yolk index = yolk height (mm) × 100/yolk width (mm),

– albumen index (%) was calculated according to the formula:

albumen index = thick albumen height (mm) × 100/albumen width (mm),

– thick albumen pH using a CPC-505 pH-conductivity meter.

– haugh units (points) were calculated according to the formula (Haugh, 1937):

$HU = 100 \log (h + 7.7 - 1.7\, W^{0.37})$, where h – thick albumen height, evaluated using a three-legged micrometer screw; W – egg weight.

Eggshell physical parameters

The number of eggs taken to analyze eggshell characteristics was 10 for each group.

Eggshell thickness (mm) was measured with the shell membranes intact, using the average of three measurements (equator, sharp end, blunt end) via a screw micrometer (Mitutoyo, 1 µm accuracy).

Elastic deformation and breaking strength were measured using a TA.XT Plus Texture Analyzer (Stable Micro Systems) with appropriate starters.

To analyze the breaking strength of the eggshell, the pressure was applied gradually until the shell cracked. This measurement defined the mass (kg) needed to break, crush, or puncture the shell.

Elastic deformation (mm) was measured with 0.001 mm accuracy under two different loads: 500 and 1000 g. This study allowed for the determination of the degree of elastic deformation of the eggshell under the influence of the applied pressure.

Biochemical composition of yolk, albumen, and shell

Thirty eggs from each group were selected to determine the chemical composition, fatty acid content, and elements in the yolk and albumen. For the chemical composition, lysozyme, and fatty acid content analysis, egg homogenates were used, with three eggs accounting for one sample.

Egg chemical composition

Egg composition was analyzed using various methods: water content was determined by drying at 105°C (PN-A-86509: 1994), protein content by the Kjeldahl method (N × 6.25) (PN-A-04018: 1975/Az3:2002), fat content via the Soxhlet method (PN-A-86509: 1994), and ash content by combustion in a muffle furnace at 600°C (BS-A-86509: 1994).

Lysozyme content

The quantity (μg/mL) of lysozyme in egg albumen was determined according to the methods described by Parry et al. [1965], with further modification by Leśnierowski *et al.* [1995]. Hydrolytic activity (U/mL) was measured using spectrophotometry (Metertech SP-830 plus; Nangang, Taipei, Taiwan, 2011).

Fatty acids composition

The fatty acid profile in the yolk was analyzed by extracting lipids from homogeneous yolk samples using methylene chloride and methanol. The fatty acid composition was determined using gas chromatography after the fatty acids were methylated (ISO/FDIS 17059). Fatty acid methyl esters (FAMEs) were prepared using the AOCS Method Ce 1k-07.

Macroelements and microelements concentration

Trace elements were analyzed via flame atomic absorption spectrometry (FAAS) using an Agilent AA Duo-AA280FS/AA280Z spectrometer (Agilent Technologies, Mulgrave, Victoria, Australia) with a Varian hollow-cathode lamp. Calibration curves were drawn with four replicates per element concentration.

Statistical analyses

The experiment used a completely randomized design. Normality was assessed using the Shapiro-Wilk test to determine if the data followed normal distributions. Bartlett's test was adopted to evaluate the homogeneity of variance. Duncan's multiple range post-hoc test was used to assess the significance of differences among groups at a significance level of $p \leq 0.05$ after ANOVA. For nonnormally distributed data, the Kruskal-Wallis test was used, followed by Dunn's test with Benjamini-Hochberg adjustments for multiple comparisons. When homogeneity of variances was violated, the Welsh One-Way ANOVA and Games-Howell's post-hoc test were applied. All analyses were performed using RStudio (2024.04.0+735, RStudio, Inc., Boston, USA).

Results and discussion

Egg physical traits and lysozyme concentration

The results regarding egg physical characteristics and lysozyme content and activity are presented in Table 1. The heaviest eggs were found in the hazy shell group (H). The weight difference between the hazy and dotted shell (D) eggs was 0.66 g. Eggs in group H were also the most elongated, as they had the lowest shape index. Heavier eggs might be expected to be more elongated to facilitate their passage through the female reproductive tract. However, Aryee *et al.* [2020] did not confirm this in quails, noting that the shape index of eggs across different weight groups was similar ($p \geq 0.05$). In a separate study, Hassan *et al.* [2013a] found no differences in the weight and shape of Japanese quail eggs at 4 months of age, regardless of shell color. Notably, the average egg weight in their study (12.7 g) was higher than that observed in the current study. Drabik *et al.* [2020] found weight differences between brown-spotted and blue eggs, with the latter being lighter (10.88 vs 10.27 g), but observed no significant differences in shape. In the present study, no differences ($p \geq 0.05$) were found in other physical traits (Tab. 1), except for the yolk index, which was significantly higher in group D. The difference between group D and the others was 4.63 percentage points. Drabik *et al.* [2020] reported a higher yolk index in blue-shelled eggs than in brown-spotted ones (41.52 vs 38.01). Similarly, Hassan *et al.* [2013a] found no differences in physical traits among quail eggs of various shell colors (light, dotted, spotted, and dark), except for the percentage of the shell. Alaşahan *et al.* [2015] also did not observe differences in the basic morphological characteristics of quail eggs with different shell colors, including shape, albumen, and yolk weight and percentage. However, they did note a higher albumen index and Haugh unit in dotted eggs compared to blue ones.

Differences in lysozyme content and activity were found between eggs with different shell colors (Tab. 1). Dotted eggs exhibited the highest average values for these traits. Although the literature lacks direct comparisons for quail eggs, Rathnasamy *et al.* [2014] reported similar lysozyme activity in quail eggs (49411 U/mg), comparable to that observed in this study. They also noted an increase in lysozyme activity with increasing pH during extraction. In the present study, significant differences in lysozyme content and activity were observed despite the albumen's pH being almost identical across groups. Albumen with higher lysozyme content and activity may characterize eggs with lower-quality shells, potentially indicating a higher natural protective barrier [Kożuszek *et al*. 2009b]. However, the findings in this study do not allow for similar conclusions regarding shell quality in quail eggs with different shell colors.

Table 1. Characteristics of egg physical traits and lysozyme content in albumen depending on shell color

Trait	Eggshell color group						*p-value*
	dotted (n=32)		spotted (n=35)		hazy (n=30)		
	mean	SEM	mean	SEM	mean	SEM	
Egg weight (g)	10.44^{b}	0.12	10.73^{ab}	0.14	11.10^{a}	0.14	0.003
Egg shape index (%)	74.47^{ab}	0.61	75.60^{a}	0.56	73.41^{b}	0.78	0.048
Yolk proportion (%)	35.25	1.25	38.22	1.09	37.57	0.93	0.152
Yolk index (%)	41.37^{a}	0.60	37.65^{b}	0.89	35.83^{b}	0.89	<0.000
Albumen proportion (%)	50.02	1.85	47.43	1.34	49.62	1.69	0.497
Albumen index (%)	6.62	0.46	6.89	0.39	6.56	0.38	0.831
Thick albumen pH	9.30	0.01	9.30	0.02	9.29	0.01	0.860
Haugh units (points)	78.56	1.22	79.82	1.01	79.33	1.00	0.699
Lysozyme content (μg/ml)	2.54^{a}	0.12	2.07^{b}	0.13	2.28^{ab}	0.16	0.040
Lysozyme activity (U/ml)	54493^{a}	2635	42010^{b}	2328	49051^{ab}	3598	0.011
Eggshell proportion (%)	15.27	0.96	14.96	0.49	15.23	0.41	0.937

[abc]In rows means bearing different superscripts differ significantly at $p \leq 0.05$.

Eggshell parameters

Differences in shell quality were observed among eggs with different shell colors (Tab. 2). The thickest shell ($p \leq 0.05$) was found in hazy eggs, which likely contributed to their lower elasticity compared to other groups, although significant differences were only noted at a pressure of 500 g. Yan *et al.* [2014] reported a significant negative correlation (-0.822) between shell thickness and elastic modulus (N/mm²). No differences were observed between shell colors regarding breaking strength. The highest calcium (Ca) content was found in the spotted group (S), with an average difference of 44.33 mg/g compared to other groups.

Table 2. Characteristics of physical parameters and elements content in eggshells depending on shell color

Trait	Eggshell color group						p-value
	dotted (n=10)		spotted (n=10)		hazy (n=10)		
	mean	SEM	mean	SEM	mean	SEM	
Thickness (mm)	0.177^{b}	0.008	0.178^{b}	0.006	0.203^{a}	0.006	0.011
Breaking strength (kg)	0.479	0,031	0.469	0,034	0.506	0,029	0.519
Elastic deformation at 500 g (mm)	0.062^{ab}	0.001	0.063^{a}	0.002	0.060^{b}	0.001	0.0001
Elastic deformation at 1000 g (mm)	0.106	0.002	0.105	0.003	0.109	0.003	0.345
Ca content (mg/g)	438.35^{b}	10.57	470.31^{a}	9.89	413.61^{c}	11.08	0.0001
Mg content (mg/g)	6.68^{a}	0.322	5.27^{b}	0.286	4.92^{b}	0.334	0.0001

[abc]In rows means bearing different superscripts differ significantly at $p \leq 0.05$.

Interestingly, hazy eggs had the lowest ($p \leq 0.05$) Ca and Mg content despite having the thickest shells. Based on these results, it is difficult to establish clear linear relationships between shell thickness, breaking strength, and trace element content. It is noteworthy that the ultrastructure of quail eggs depends on shell color. Hassan *et al.* [2013b] found that the palisade and mammillary layers were less defined in light-colored shells. The thickest mammillary layer was observed in dark-shelled eggs, identified as spotted in this study. Studies on pheasant eggs have shown that blue eggshells had a shallower mammillary layer. Moreover, excessive contact between the papillae in this layer and the shell membrane suggested a lack of cohesion, indicating an abnormal shell structure [Krystianiak *et al.* 2005].

Basic chemical composition

The highest dry matter content was found in spotted eggs (Tab. 3), while hazy eggs had the highest crude protein content, with a difference of 0.55 percentage points compared to other groups. Hazy eggs also had the lowest crude fat and crude ash content. Dudusola *et al.* [2010] reported similar values for crude protein and fat in Japanese quail eggs but found lower crude ash content (1.04%) compared to this study. The lower crude ash content in hazy eggs is likely due to their lower macroelement and microelement content (Tab. 5). Nowaczewski *et al.* [2013] reported for pheasant eggs that blue eggs had lower element content.

Table 3. Basic chemical composition (%) of whole egg (yolk and albumen) depending on shell color

Trait	Eggshell color group						*p-value*
	dotted (n=10)		spotted (n=10)		hazy (n=10)		
	mean	SEM	mean	SEM	mean	SEM	
Dry matter	26.9[b]	0.160	27.8[a]	0.283	26.8[b]	0.208	0.013
Crude protein	12.7[b]	0.057	12.6[b]	0.069	13.2[a]	0.110	<0.001
Crude fat	11.8[b]	0.117	12.8[a]	0.267	11.4[b]	0.267	<0.001
Crude ash	1.48[a]	0.035	1.41[a]	0.046	1.23[b]	0.046	<0.001

[abc]In rows means bearing different superscripts differ significantly at p≤0.05.

Table 4. Fatty acid composition (%) of egg yolk depending on shell color

Trait	Eggshell color group						*p-value*
	dotted (n=10)		spotted (n=10)		hazy (n=10)		
	mean	SEM	mean	SEM	mean	SEM	
C14:0	0.583	0.021	0.560	0.033	0.535	0.014	0.381
C16:0	36.491	0.393	36.849	0.135	36.352	0.342	0.515
C16:1	4.682[a]	0.081	4.429[ab]	0.077	4.007[b]	0.233	0.012
C18:0	21.892	0.438	23.420	1.695	20.138	0.193	0.092
C18:1	18.225[b]	0.334	17.603[b]	1.354	21.41[a]	0.358	0.007
C18:2	16.024[a]	0.210	15.108[b]	0.234	15.498[ab]	0.066	0.006
C18:3	0.497	0.012	0.509	0.014	0.515	0.019	0.703
C20:0	0.267	0.016	0.250	0.016	0.232	0.009	0.240
C20:4	1.041	0.013	0.980	0.039	1.009	0.037	0.411
MUFA	22.907[b]	0.259	22.032[c]	1.382	25.417[a]	0.162	0.019
PUFA	17.562[a]	0.202	16.597[b]	0.211	17.022[b]	0.071	0.002
SFA	59.233[ab]	0.123	61.079[a]	1.577	57.257[b]	0.203	0.024

[abc] In rows means bearing different superscripts differ significantly at p≤0.05.

Table 5. The content of macro- and microelements (mg/kg) in eggs interior depending on shell color

Trait	Eggshell color group						*p-value*
	dotted (n=10)		spotted (n=10)		hazy (n=10)		
	mean	SEM	mean	SEM	mean	SEM	
Magnesium	365[a]	8.89	304[b]	10.3	284[b]	9.58	<0.001
Calcium	2147[a]	97.1	1540[b]	98.3	1232[c]	97.1	<0.001
Selenium	0.35[b]	0.008	0.28[c]	0.007	0.36[a]	0.008	<0.001
Copper	0.58[a]	0.035	0.17[b]	0.042	0.19[b]	0.028	<0.001
Manganese	0.76[a]	0.009	0.66[c]	0.008	0.70[b]	0.009	<0.001
Pottasium	2018[a]	24.3	1818[b]	25.4	1 997[a]	22.1	<0.001
Sodium	2828[a]	105.5	1787[b]	107.3	2 158[b]	98.2	<0.001
Zinc	13.9[b]	0.80	14.4[b]	0.78	20.2[a]	0.75	<0.001
Iron	40.7[a]	1.84	45.5[a]	1.92	58.6[b]	1.88	<0.001

[abc] In rows means bearing different superscripts differ significantly at p≤0.05.

Fatty acid composition

Changes in fatty acid content and profile in egg yolk are often attributed to dietary modifications. Studies on this topic in Japanese quail have been conducted by Kaźmierska *et al.* [2007], da Silva *et al.* [2009], and Trziszka *et al.* [2014]. However, age-related changes in fatty acid content have also been demonstrated in quail eggs [Nowaczewski *et al.* 2021]. In this study, differences were observed for palmitoleic acid (C16:1), oleic acid (C18:1), and linoleic acid (C18:2) between the egg groups (Tab. 4). Dotted eggs had the highest levels of C16:1 and C18:2. Lower levels of oleic acid were found in both dotted and spotted eggs compared to hazy eggs, with an average difference of 3.5 percentage points. Dotted eggs also had a higher PUFA content than the other groups (17.56 vs. 16.81%). Trziszka *et al.* [2014] found the highest oleic acid content (C18:1) in Japanese quail eggs, ranging from 31.17% to 43.84%. In this study, palmitic acid (C16:0) was the most abundant fatty acid in the profile. Polat *et al*. [2013] found higher levels of C18:1 (39.9%) and C18:2 (22.2%) in quail eggs (*Coturnix coturnix*).

The presence of oleic and linoleic acids in animal products like eggs is significant, as they are associated with reduced total cholesterol levels, increased HDL levels, and reduced inflammation [Pravst 2014]. Polyunsaturated fatty acids (PUFA) play a key role in lowering blood triglyceride levels, reducing blood pressure, and preventing blood clots. Therefore, higher levels of these fatty acids in animal products like eggs and meat are beneficial. Based on the present study, dotted eggs had the most favorable fatty acid composition, particularly regarding PUFA content, while spotted eggs had the least favorable profile.

Macroelements and microelements

Eggs (yolk and albumen) are rich in Na, P, Cl, K, S, Ca, Mg, and Fe, while trace elements such as Zn, F, Br, I, Cu, Mn, Ar, Cr, Al, and Co are present in small amounts [Giannenas *et al.* 2009, Bouvarel *et al.* 2010]. The differences in macroelement and microelement content were observed among the egg groups with different shell colors (Table 5). From a nutritional perspective, dotted eggs contained the highest levels of Mg, Ca, Cu, Mn, K, and Na, while they had the lowest Zn and Fe levels. Similar findings on element content differences related to shell color have been reported in pheasant eggs [Nowaczewski *et al*. 2013]. Dark brown-shelled eggs contained the highest levels of K, Cu, Ca, and Mg, with Na content higher by 188 and 116 mg/L compared to light brown and olive eggs, respectively.

Eggshell coloration is primarily influenced by three primary pigments: protoporphyrin-IX, biliverdin-IX, and zinc biliverdin chelate [Sparks 2011]. Protoporphyrin-IX produces a brown shell color, while biliverdin-IX and zinc biliverdin chelate are responsible for blue and green shell colors [Zhao *et al*. 2006]. Because no studies on the zinc content in Japanese quail eggshells were found in the literature, it would be worth considering this aspect when planning further studies.

Conclusion

Although the dotted eggs were the smallest, they had the highest lysozyme content in the egg albumen, which could significantly contribute to their longer freshness.

Additionally, these eggs were characterized by a higher proportion of PUFA and a greater content of the analyzed macroelements and microelements compared to other groups, likely resulting in the highest amount of crude ash in dotted eggs. However, no relationship was found between eggshell thickness and resistance to crushing or macroelement content.

In conclusion, dotted eggs may seem more attractive to consumers due to their favorable physical and biochemical properties.

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