

Interrelationships between Morphological, Densitometric and Mechanical Properties of Eggs in Japanese Quails (*Coturnix Japonica*)

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Eggshell quality in birds results from mineral density and composition determining its mechanical endurance. The aim of the study was to determine interrelationships between morphological, densitometric and mechanical properties of eggs in Japanese quails. Twenty four eggs randomly collected from 17-week-old quails were subjected to morphological, densitometric and mechanical evaluation using dual-energy X-ray absorptiometry (DEXA), quantitative computed tomography (QCT) and three-point bending test. Weight, height and width of eggs were positively correlated with the densitometric parameters obtained using DEXA (egg mineral density (EMD) and egg mineral content (EMC)) and QCT (total egg volume (TEVol) and total eggshell volume (TESVol)). Positive correlations were stated between TEVol and TESVol ($r=0.52$; $P<0.05$) and EMD and EMC ($r=0.83$; $P<0.05$). Egg mineral density revealed positive correlations with TEVol and mean volumetric eggshell mineral density (MvESMD), while EMC was positively correlated with TEVol, TESVol and MvESMD (all $P<0.05$). Eggshell breaking strength was positively correlated with MvESMD ($r=0.53$; $P<0.05$) and negatively correlated with eggshell thickness ($r=-0.50$; $P<0.05$). In conclusion, the results obtained in this study showed numerous interrelationships between morphological, densitometric and mechanical properties of eggs in Japanese quails. Both DEXA and QCT were shown to be valuable tools for evaluation of whole egg and eggshell quality with superior prognostic value of QCT for eggshell mechanical endurance prediction. The elaborated experimental model may serve for further investigations on physiological, pharmacological, environmental, nutritional and toxicological factors influencing egg quality.

Key words: densitometry, dual-energy X-ray absorptiometry (DEXA), egg, Japanese quail, quantitative computed tomography (QCT)

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Introduction

Egg quality in birds results from nutritional value of egg yolk constituents and eggshell mineral composition determining its mechanical endurance (Solomon, 2010; Abbaspour *et al.*, 2015). Eggshell consists of 97% calcium carbonate and forms an embryonic chamber for the developing embryo, ensuring its protection from mechanical damage. Eggshell

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regulates gas exchange between the developing embryo and the external environment, and prevents contamination by bacterial and other pathogens. Eggshell provides also the metabolic function for developing embryo serving as a reservoir of nutrients, especially calcium (Hunton, 2005). Calcite is the dominant morphological form of calcium carbonate in egg; however, vaterite and aragonite contribute to the eggshell composition (Solomon and Bain, 2012). Intact structure and good quality of eggshell resulting from mineral density limit bacterial penetration into albumen and yolk. Small defects of poorly mineralized eggshell occurring during egg handling procedures facilitate movement of bacteria into the egg contents (Messens *et al.*, 2005; De Reu *et al.*, 2006; Roberts *et al.*, 2013). Thus, eggshell provides optimal package for an important food item. Mechanical failure of eggshell structure decreases egg's value leading to poor economic outcomes due to the loss of nutritional value and increased embryo mortality (Hunton, 2005). Considering importance of proper structure and mineral composition of eggshell for protective functions and whole egg quality, the aim of the study was to determine interrelationships between morphological, densitometric and mechanical properties of eggs from Japanese quails (*Coturnix japonica*).

Material and Methods

The experimental procedures used in this study were approved by The 3rd Local Ethics Committee on Animal Experimentation in Warsaw (SGGW Warsaw) in a Resolution No 27/2009 and performed in accordance with the Guiding Principles for the Care and Use of Research Animals.

Experimental Design

The study was performed on 24 eggs randomly collected from three groups of 17-week-old Japanese quails. The birds used in the experiment were the 9th generation of Japanese quails fed genetically modified or control diet. Each generation of quails was kept in cages in standard rearing conditions (recommended environmental temperature for Japanese quails and 14:10 hrs light:dark cycle) until the 17th week of life to collect fertilized eggs for incubation to obtain the next quail generation. Feed composition and nutritional value of feeds used in the experiment are presented in Table 1. To the control group belonged quails receiving standard diet recommended for several periods of growth (1–6 week) and laying (7–17 week) without genetically modified (GM) ingredients. In the second group (Ex I group) GM soybean

Table 1. Feed composition and nutritional value of feeds used during the experiment in three groups of Japanese quails

Indices	Grower feed (1–6 week)			Layer feed (7–17 week)		
	Group			Group		
	Control	Ex I	Ex II	Control	Ex I	Ex II
<i>Ingredients, g/kg</i>						
Soya bean meal non-GMO	390.0	0.0	390.0	295.0	0.0	295.0
RR soya bean meal GMO	0.0	390.0	0.0	0.0	295.0	0.0
Maize grain MON 810 GMO	0.0	0.0	250.0	0.0	0.0	250.0
Maize grain non-GMO	250.0	250.0	0.0	250.0	250.0	0.0
Wheat	196.7	196.7	222.0	233.8	233.3	233.8
Triticale	25.0	25.0	0.00	50.0	50.0	50.0
Rapeseed meal	41.7	41.7	41.7	0.0	0.0	0.0
Rape seed	0.0	0.0	0.0	55.6	55.6	55.6
Soya oil	22.3	22.3	21.9	30.0	29.8	30.0
Potato protein	25.3	25.3	23.1	0.0	0.0	0.0
Maize gluten	15.5	15.5	17.8	0.0	3.1	0.0
Limestone	13.4	13.4	13.4	62.6	62.6	62.6
Monocalcium phosphate	9.0	9.0	9.0	9.5	9.5	9.5
Additives ¹	11.1	11.1	11.1	13.5	11.1	13.5
<i>Nutritional value (calculated)</i>						
AME, kcal/kg	2950	2950	2950	2800	2800	2800
Crude protein, %	26.50	26.50	26.50	20.84	20.86	20.84
Crude fat, %	6.10	6.10	6.04	5.56	5.54	5.56
Crude fibre, %	2.79	2.79	2.80	3.01	3.01	3.01
Crude ash, %	5.85	5.85	5.85	10.39	10.39	10.39
Calcium, %	0.97	0.97	0.97	2.77	2.78	2.77
Available P, %	0.45	0.45	0.45	0.45	0.45	0.45

¹ The following additives were included in the diets in grower/layer feed (g/kg): vitamin-mineral premix 3.2/5, NaCl 2.5/2.5, sodium bicarbonate 1.4/2.1, phyzyme 0.1/0.1, ronozyme 0/0.2, methionine 1.8/1, L-lysine 0/0.4, and threonine 0/2.3
AME - Apparent Metabolic Energy

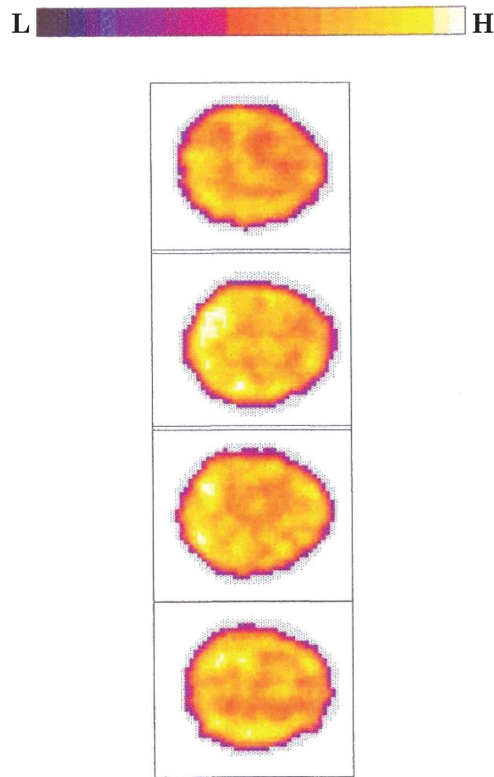


Fig. 1. **Dual-energy X-ray absorptiometry of eggs collected from 17-week-old Japanese quails.** Egg mineral density (EMD) and egg mineral content (EMC) were determined for each egg in the lateral direction using Norland XR-46 apparatus supplied with Research Scan software. The measuring scan dimension was set at 1.0×1.0 mm.

meal was provided. Genetically modified soybean meal was produced from a Rundup Ready soybean (*Glycine max. L cv A 5403*). In the third group (Ex II group) GM maize grain was included. Genetically modified maize grain was MON 810 maize with specific *Bt* (*Bacillus thuringiensis*) gene construct. Feeds used during the experiment were prepared by Agro-Kocięba company (Agro-Kocięba, Czarnocin, Poland). Basic nutrient contents in the feeds used in the experiment were analyzed in accordance to the method provided by Association of Official Agricultural Chemist (AOAC, 2005) and the compositions of the feeds were confirmed to be as planned.

Morphometric and Densitometric Evaluation of Eggs

Collected eggs were weighted immediately after laying on precise electronic balance PS 8000/C/1 with an accuracy 0.01 g (Radwag, Radom, Poland). Egg height and egg width were measured using a vernier caliper. Egg mineral density (EMD) and egg mineral content (EMC) were determined for each egg in the lateral direction using dual-energy X-ray absorptiometry (DEXA) method and Norland XR-46 apparatus supplied with Research Scan software (Norland, Fort Atkinson, WI, USA). The measuring scan dimension was set at 1.0×1.0 mm (Fig. 1). Quantitative computed tomography

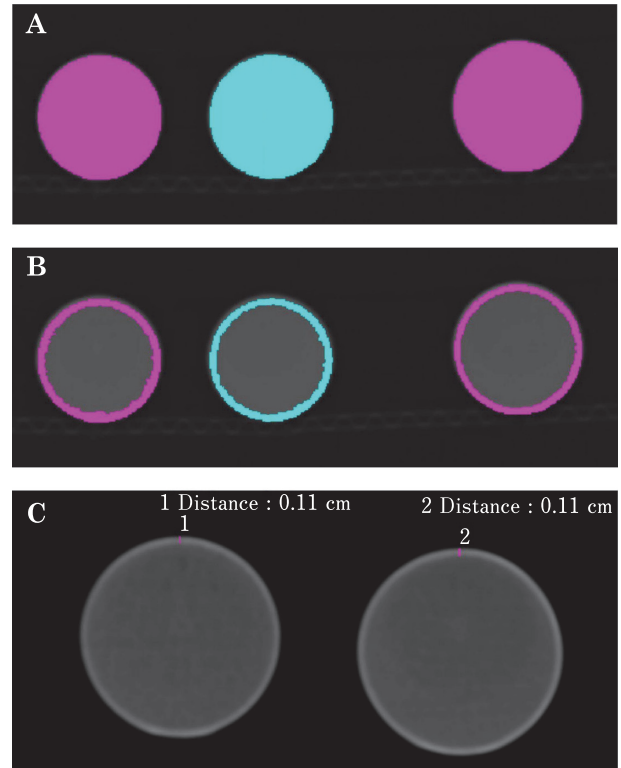


Fig. 2. **Measurements of mean volumetric egg mineral density - MvEMD (A), mean volumetric eggshell mineral density - MvESMD (B) and eggshell thickness (C) using quantitative computed tomography (QCT) method.** For MvEMD of whole egg and MvESMD determinations, eggs samples were scanned in transversal plane (A-P direction) using 1-mm thick scans. The volume-of-interest (VOI) for MvEMD and total egg volume (TEvol) measurements were defined by limiting the minimum and maximum density of the investigated egg at -25 and 3071 Hounsfield units (HU), respectively (A). For MvESMD and total eggshell volume (TESvol) calculations, the VOI was limited between the minimum and maximum density of the investigated egg at 55 and 3071 HU, respectively (B). 1-mm thick, cross-sectional QCT scan placed at 50% of egg sample length was used for the automatic measurement of eggshell thickness (C).

(QCT) method and Somatom Emotion Siemens apparatus (Siemens, Erlangen, Germany) supplied with Somaris/5 VB10B software were used for mean volumetric egg mineral density (MvEMD) determination of whole egg (Fig. 2A) as well as for mean volumetric eggshell mineral density (MvESMD) determination, including whole eggshell exclusively (Fig. 2B). Using this method, total egg volume (TEvol) and total eggshell volume (TESvol) were determined. All eggs were scanned in transversal plane (A-P direction) using 1-mm thick scans. The values of MvEMD, MvESMD, TEvol and TESvol were determined using Volume Evaluation application package (Software Version B10/2004A). For TEvol and MvEMD calculations, the

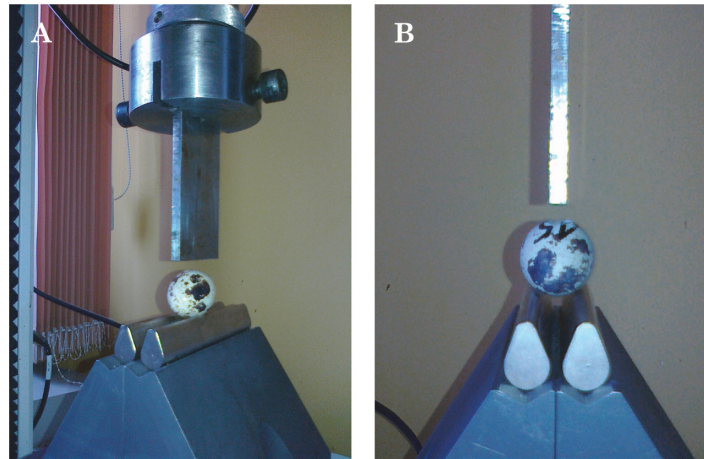


Fig. 3. Eggshell breaking strength determination using three-point bending test in eggs obtained from 17-week-old Japanese quails. Single egg was placed in accordance to its longitudinal axis on semi-circular supports and plane-shape measuring head loaded the sample on convex surface of eggshell with a constant speed of 50 mm/min. The distance between egg supports was set at 1.5 cm in length (A). Egg sample placement on the circular supports in accordance to its longitudinal axis enabled eggshell crushing directly by the loading head and precise determination of eggshell breaking strength that mimics eggshell crushing during egg laying or handling procedures. In all egg samples, the measuring head crushed the eggshell surface that was untouched in the area adjacent to the egg supports (B).

volume-of-interest (VOI) was defined by limiting the minimum and maximum density for the investigated egg at -25 and 3071 Hounsfield units (HU), respectively. For TESvol and MvESMD calculations, the VOI was limited between the minimum and maximum density for the investigated egg at 55 and 3071 HU, respectively. Moreover, using 1-mm thick, cross-sectional QCT scan, placed at 50% of each egg length, the thickness of eggshell at this place was measured automatically. Eggshell thickness was measured in a single measuring point, since it was not differentiated around whole cross-section of egg (Fig. 2C).

Mechanical Testing of Eggs

Eggshell breaking strength was determined for each egg in an Instron 3367 apparatus (Instron, Canton, MA, USA). Three-point bending test was used. Single egg was placed in accordance to its longitudinal axis on semi-circular supports and plane-shape measuring head loaded the sample on convex surface of shell with a constant speed of 50 mm/min. The distance between egg supports was set at 1.5 cm in length (Fig. 3A). Such position of egg sample on the semi-circular supports enabled eggshell crushing directly by the loading head in all cases and precise determination of eggshell breaking strength that mimics structural eggshell disintegration during egg laying or handling procedures and cracks incidence. In all egg samples, the measuring head crushed the eggshell surface that was untouched in the area adjacent to the egg supports (Fig. 3B).

Statistical Analysis

Statistical analysis of data collected was performed using Statistica software (version 6.0). Pearson's correlation coefficient (r) was determined between all the investigated parameters of eggs. P -value < 0.05 was considered as statistically significant for all the obtained correlations.

Results

The results of morphological, densitometric and mechanical evaluations of eggs obtained from Japanese quails are shown in Table 2.

The values of Pearson correlation coefficient estimated between all the investigated parameters of eggs are presented in Table 3. Egg weight was positively correlated with such parameters as egg height, egg width, TEvol, EMD, EMC and TESvol ($P < 0.05$). Egg height was positively correlated with egg width ($P = 0.003$) and both these parameters were positively correlated with TEvol, EMD and TESvol ($P < 0.05$). Total egg volume was also positively correlated with EMD, EMC and TESvol, while EMD was positively correlated with EMC and MvESMD ($P < 0.05$). Positive correlations of TESvol and MvESMD with EMC and MvEMD was stated ($P < 0.05$). Moreover, eggshell breaking strength was positively correlated with MvESMD ($P = 0.007$) and negatively correlated with eggshell thickness ($P = 0.01$).

Discussion

Considering that detailed examination of eggs from laying

Table 2. Morphological, densitometric and mechanical properties of eggs (N=24) collected from Japanese quails at the age of 17 weeks of life

Investigated parameter	Mean value	Range
Egg weight (g)	12.84±0.21	10.05–14.86
Egg height (mm)	33.50±0.26	31.70–35.90
Egg width (mm)	26.47±0.15	24.60–27.70
Total egg volume (cm ³)	11.71±0.19	9.48–13.56
Egg mineral density (g/cm ²)	0.137±0.001	0.124–0.149
Egg mineral content (g)	1.18±0.02	1.026–1.373
Mean volumetric egg mineral density (g/cm ³)	1.064±0.002	1.050–1.078
Eggshell volume (cm ³)	3.22±0.08	2.29–3.98
Mean volumetric eggshell mineral density (g/cm ³)	1.157±0.003	1.126–1.186
Eggshell thickness (mm)	0.89±0.02	0.80–1.10
Eggshell breaking strength (N)	13.05±0.40	9.33–16.76

Values are means±SEM.

Table 3. The values of Pearson's correlation coefficient estimated between all the investigated variables of eggs from Japanese quails

Investigated parameter	Egg weight	Egg height	Egg width	Egg volume	Egg mineral density	Egg mineral content	Mean volumetric egg mineral density	Eggshell volume	Mean volumetric eggshell mineral density	Eggshell thickness	Eggshell breaking strength
Egg weight	x	0.80***	0.91***	0.87***	0.41*	0.83***	0.29	0.60**	0.30	0.14	0.16
Egg height	0.80***	x	0.58**	0.67***	0.18	0.64**	0.28	0.54**	0.22	0.07	0.20
Egg width	0.91***	0.58**	x	0.77***	0.33	0.69***	0.13	0.42*	0.15	0.16	0.03
Egg volume	0.87***	0.67***	0.77***	x	0.45*	0.78***	0.14	0.52**	0.28	0.33	-0.01
Egg mineral density	0.41*	0.18	0.33	0.45*	x	0.83***	0.39	0.26	0.74***	0.26	0.30
Egg mineral content	0.83***	0.64***	0.69***	0.78***	0.83***	x	0.39	0.51*	0.62**	0.23	0.28
Mean volumetric egg mineral density	0.29	0.28	0.13	0.14	0.39	0.39	x	0.80***	0.54**	-0.19	0.28
Eggshell volume	0.60**	0.54**	0.42*	0.52**	0.26	0.51*	0.80***	x	0.20	-0.09	-0.02
Mean volumetric eggshell mineral density	0.30	0.22	0.15	0.28	0.74***	0.62**	0.54**	0.20	x	0.15	0.53**
Eggshell thickness	0.14	0.07	0.16	0.33	0.26	0.23	-0.19	-0.09	0.15	x	-0.50*
Eggshell breaking strength	0.16	0.20	0.03	-0.01	0.30	0.28	0.28	-0.02	0.53**	-0.50*	x

Significance levels: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

birds may be utilized in experimental studies focused on regulatory processes of mineral, protein and lipid metabolism, as well as female reproductive system functions, it must be indicated that very limited information are available on usefulness of QCT and DEXA techniques for assessment of egg quality. This study for the first time presents use of both QCT and DEXA for investigations of morphological and densitometric properties of egg in Japanese quails and their common relationships. Both these methods are commonly used for non-invasive *in vivo* or *ex vivo* determinations of quality of teeth and bones of skeletal system in mammals and birds (Charuta *et al.*, 2008; Tatara *et al.*, 2011; Charuta and Cooper, 2012; Tymczyna *et al.*, 2012; Tymczyna *et al.*, 2013). In case of both these methods, morphological imaging and densitometric measurements are based on data ob-

tained from X-rays attenuation during passage throughout the examined object. X-rays undergo physical attenuation that is proportional to the density of the medium included to the region or volume of interest. DEXA method measures areal mineral density of the object that is expressed in g/cm² in two-dimensional and size-dependent manner. Moreover, using DEXA method, mineral content of the examined sample can be determined in grams (Guglielmi *et al.*, 1994; Tatara, 2006). As shown in the current study, both EMD and EMC can be routinely determined in eggs collected from Japanese quails. As opposite to DEXA method providing mainly data related to egg mineralization, the determination of both morphological and densitometric properties of the examined samples can be performed using QCT method (Krupski and Tatara, 2007). In this study, QCT method was

used to determine morphological and densitometric characteristics for both whole egg and eggshell exclusively. Using QCT, eggshell was examined in terms of eggshell thickness, TESvol and MvESMD, while for the whole egg, TEvol and mean MvEMD were determined. As shown in previous studies on eggs, whole egg quality was determined mainly using such parameters as egg weight, egg mass, albumen weight/content, yolk weight/content, yolk and shell colour, shell weight and Haugh unit (Świątkiewicz and Koreleski, 2007; Mejia *et al.*, 2010; McNaughton *et al.*, 2011; Roberts *et al.*, 2013). Basic constituents such as water, proteins, carbohydrates, fats and minerals were also determined in eggs (Ren *et al.*, 2010). To investigate eggshell quality, eggshell thickness, weight, breaking strength, elemental composition and microarchitectural arrangement were analyzed (Świątkiewicz and Koreleski, 2007; Hincke *et al.*, 2010; Roberts *et al.*, 2013; Stefanello *et al.*, 2014). Moreover, palisade and mammillary layers thickness, percentage of broken eggs or shell crack numbers were determined (Radwan *et al.*, 2010; Gheisari *et al.*, 2011; McNaughton *et al.*, 2011). Even though previously elaborated methods of egg evaluation provide valuable data, non-destructive radiological methods for qualitative and quantitative evaluation of both eggshell and whole eggs were not reported, especially when one considers common interrelationships between these parameters.

In the current study, it was shown that morphological traits such as egg weight, height and width, which were measured with a use of traditional methods, correlate positively with the densitometric parameters obtained using DEXA (EMD and EMC) and QCT (TEvol and TESvol) techniques. Strong positive interrelationships were stated between the parameters determined using QCT (TEvol and TESvol; $r=0.52$) as well as DEXA method (EMD and EMC, $r=0.83$). Moreover, values of EMD revealed positive correlations with TEvol and MvESMD, while EMC was found to be positively correlated with TEvol, TESvol and MvESMD values, indicating that both these radiological techniques may be proposed as valuable tools for investigations on the factors affecting egg quality in birds. Comparing the predictive value of both these techniques for egg quality determination, the superior precision can be ascribed to QCT than to DEXA due to the fact that MvESMD was found to be positively correlated with eggshell breaking strength ($r=0.53$). Neither EMD nor EMC were found to be significantly correlated with the mechanical endurance of eggshells. The obtained results revealed also that eggshell thickness is negatively correlated with eggshell breaking strength indicating that mechanical endurance of the eggshell is not simply affected by its thickness but other factors such as mineral density, mineral content and spatial microarchitectural arrangement contribute to this characteristic. A contribution of the presence of eggshell matrix proteins to mechanical endurance of egg can not be excluded; however, this issue requires further studies to be better understood (Hincke *et al.*, 2010). Regardless the factors influencing mechanical endurance of eggshells, methodological approach used in this study to the

testing mechanical endurance of eggs may be applied to eggs collected from other bird species. Semicircular shape of egg supports used in three-point bending test during the mechanical testing of eggs, egg placement on the supports in accordance to its longitudinal axis, and application of plane-shape measuring head ensure independent on egg size and precise determination of eggshell breaking strength. Thus, such experimental settings enables mechanical endurance testing of eggs obtained from various bird species in conditions mimicking structural eggshell crushing during laying and routine handling procedures. Differentiated egg size from various bird species in this settings seems not to be a serious limitation of the mechanical testing of eggshell since the distance between the egg supports may be flexibly regulated.

In conclusion, the results obtained in the current study showed numerous interrelationships between morphological, densitometric and mechanical properties of eggs collected from Japanese quails. Both DEXA and QCT methods were shown to be precise and valuable tools for evaluation of whole egg and eggshell quality. The superior prognostic value in determination of egg quality may be ascribed to QCT since MvESMD measured with this technique was found to be positively correlated with mechanical endurance of eggshell. The elaborated experimental model in this study may serve for further investigations on physiological, pharmacological, environmental, nutritional and toxicological factors influencing egg quality not only in Japanese quails but in other bird species as well.

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