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Effect of age of Japanese quail on physical and biochemical characteristics of eggs

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Abstract

The aim of the study was to investigate the effects of age of birds on egg quality in Japanese quail. The eggs were randomly selected from among all eggs laid on the same day when the birds were 15, 23 and 31 weeks old. At each time point, 90 fresh eggs were evaluated for their physical and biochemical characteristics. Egg weights were similar over time. At 23 and 31 weeks, the eggs had less shell than at 15 weeks. Crude fat and ash contents of the eggs increased with the age of the birds. Crude protein was also highest in eggs of the oldest quail. At 31 weeks old, the eggs were lowest in pH of yolk and white. Quail that were23 and 31 weeks old laid eggs with significantly higher polyunsaturated fatty acid (PUFA) and lower saturated fatty acid (SFA) contents. The lowest cholesterol content was in egg yolks from 23-week-old quail. The oldest birds had the highest contents of sodium, potassium, zinc, selenium, copper, and manganese. The content and activity of lysozyme decreased with ageing of the birds. From the consumers' point of view, eggs from older birds appeared to be the most valuable. At the same time, as the quail ages, the antibacterial properties of eggs deteriorate, which may indicate a shorter shelf life.

Keywords: age effects, *Coturnix coturnix japonica*, egg quality, poultry
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Introduction

Although hen eggs dominate the egg market, in some countries Japanese quail eggs have a significant share. The popularity of these eggs may be in part because of the large proportion of yolk, often over 30% (Hrnčár *et al.*, 2014). However, it seems that the most important thing is that quail egg protein is considered hypoallergenic (Benichou *et al.*, 2014). Therefore, these eggs may be an alternative for people that are allergic to chicken egg protein. With constantly growing interest in quail eggs and meat, the high hatchability results of these species will be an important breeding strategy. Unfortunately, the literature indicates that the hatchability of quail is seldom satisfactory and is quite variable. The hatchability of fertilised eggs may range from 50.3% to 90.4% (Seker *et al.*, 2005; Nowaczewski *et al.*, 2012; Alasahan & Copur, 2016).

Physical traits and the quality of internal (yolk and white) and external characteristics of poultry eggs affect hatchability and the subsequent performance of the chicks (Ulmer-Franco, 2010; Alabi *et al.i* 2012; Terčič & Smerdu, 2015). Biochemical characteristics of eggs, including cholesterol levels, macro- and micro-mineral content, and pH of white, can also affect hatchability (Speake *et al.*, 1998; Perelman *et al.*, 2001; Yilmaz Dikmen & Sahan 2007; Reijrink *et al.*, 2008). The amount and activity of lysozyme in egg can be important information for both table and hatching eggs. Hen egg white lysozyme is an alkaline globular protein that is characterized by high enzymatic activity, primarily bactericidal and fungicidal, but lysozyme can inactivate viruses (Vilcacundo *et al.*, 2018).

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Egg weight usually increases as females age, whereas characteristics of the egg white, eggshell quality and hatchability deteriorate (Akyurek & Okur 2009; Abudabos *et al.*, 2017; Nasri *et al.*, 2020). Thus, the effects of the age of quail hens on egg quality should not be overlooked. The scientific literature contains basic assessments of egg yolks, whites and eggshells related to the age of birds (Adamski, 2008; Nhan *et al.*, 2018). However, few data are related to the biochemical characteristics of eggs of this species (Tolik, 2014; Sathya & Murugaian 2015). Further, no results could be found that discussed changes in the biochemical characteristics and internal traits of quail eggs as functions of the age of the quail hen. Thus, the aim of this study was to investigate changes in the values of physical and biochemical characteristics of eggs as Japanese quail hens aged.

Materials and methods

According to Polish law and the EU directive (no 2010/63/EU), this study did not require additional approval of the Local Ethical Committee for Experiments on Animals.

Eggs from Japanese quail *(Coturnix coturnix japonica)* hens in their first year of life were obtained from a commercial farm in Poland. Up to 6 weeks old, chicks were kept on rye or triticale litter in a 40 m^2 rearing chamber. During the growing period, Japanese quail were fed a complete diet ad libitum, which contained 12.08 MJ/kg ME, 24.0% crude protein, 7.1% crude fat, 3.0% crude fibre, and 1.0% calcium. The hens began to lay eggs when they were seven weeks old. The experiment ended when the hens were 31 weeks old. Average bodyweight of females at seven weeks was 148.4 ± 3.9 g. During the entire laying period, the experimental birds (540 females and 90 males) were kept in 30 cages (18 females and 3 males in each cage) at a density of 35 birds/m^2. The adult Japanese quail were fed a complete diet ad libitum, which contained 11.63 MJ/kg ME, 20.5% crude protein, 5.0% crude fat, 3.0% crude fibre, and 3.1% calcium. The diet was composed of 52.26% corn, 37.70% soybean meal, 6.08% limestone, 0.43% common salt, 0.93% soybean oil, and 3.00% vitamin-mineral premix. The 90 eggs that were used to represent each age were randomly selected from all the eggs laid on a single day when the hens were 15, 23 and 31 weeks old. The weights (g) of the yolk and white were measured with 0.01 g accuracy. Eggshell weight (g) was measured after drying at 105 °C until it reached constant weight. Percentages of yolk, white and eggshell were calculated relative to egg mass.

Polish standard methods were used to analyse water content (PN-A-86509: 1994), crude protein (PN-A-04018: 1975/Az3:2002), fat (PN-A-86509: 1994) and ash(BS-A-86509: 1994). Briefly, to determine the water content, samples were dried at 105 °C until weight stasis. Protein was measured as Kjeldahl nitrogen multiplied by 6.25. Fat content was determined by Soxhlet extraction. Ash was the amount of sample that remained after burning in a muffle furnace at 600 °C. The pH of the egg white and yolk were measured with a waterproof pH-meter (CP-401, Elmetron, Zabrze-Grzybowice, Poland).

Lipids were extracted from homogeneous yolk samples with a mixture of methylene chloride and methanol. Cholesterol content was determined by gas chromatography following the AOCS (1997) official method. Fatty acid composition was determined after methylation by gas chromatography. Methyl esters of fatty acids (FAME) were prepared according to AOCS (2007).

Concentrations of selected trace elements in eggs (mg*kg^{-1}) were analysed with the atomic absorption spectrometry (FAAS) method using a spectrometer (Agilent Technologies AA Duo - AA280FS/AA280Z, Agilent Technologies, Mulgrave, Victoria, Australia) equipped with a Varian hollow-cathode lamp.

The lysozyme content (%) and activity (U/ml) were determined with the electrophoretic method (Leśnierowski & Kijowski, 1995), whereas the hydrolytic activity of this enzyme was determined with spectrophotometry.

Statistical analyses were conducted with SAS version 9.2 (SAS Institute Inc., Cary, North Carolina, USA). Means values ($\bar{x}$) and associated standard errors were calculated for all traits. The effect of age of the bird at the time of laying was tested with one-way analysis of variance. Comparisons of the group means were done with Fisher's least significant difference test.

Results and Discussion

Egg, yolk, albumin and shell weights were similar, irrespective of the age of the hen (Table 1). Further, only the relative amount of eggshell was affected significantly with 23- and 31-week-old hens producing eggs with a lower eggshell content compared with 15-week-old quail.

Table 1 Effect of age of Japanese quail on egg characteristics

Trait	15th week	23rd week	31st week	Overall
Egg weight, g	11.59 ± 0.18	11.66 ± 0.13	11.67 ± 0.13	11.64 ± 0.15
Yolk weight, g	3.54 ± 0.09	3.56 ± 0.04	3.50 ± 0.06	3.53 ± 0.06
Yolk content, %	30.47 ± 0.36	30.52 ± 0.22	29.93 ± 0.31	30.31 ± 0.30
Albumen weight, g	7.11 ± 0.11	7.20 ± 0.09	7.25 ± 0.08	7.19 ± 0.09
Albumen content, %	61.36 ± 0.36	61.70 ± 0.22	62.17 ± 0.31	61.74 ± 0.30
Eggshell weight, g	0.95 ± 0.02	0.91 ± 0.01	0.92 ± 0.01	0.93 ± 0.15
Eggshell content, %	8.17^{a} ± 0.11	7.77^{b} ± 0.08	7.89^{b} ± 0.08	7.94 ± 0.09

^{a,b} Within a row, means with a common superscript did not differ with probability *P* =0.05

In this research, no effect that stemmed from the age of Japanese quail was observed on most of these physical characteristics of eggs, including their weight. This does not conform to the generally accepted rule that with an increase in the age of female birds, the weight of their eggs increases (Padhi *et al.*, 2013; Yamak *et al.*, 2015; Biesiada-Drzazga *et al.*, 2016). Ipek *et al.* (2004) and Nhan *et al.* (2018) confirmed this generality for Japanese quail. However, Nowaczewski *et al.* (2010) found eggs from 25-week-old Japanese quail were lighter than those from 9-week-old birds. Therefore, based on the present results and those from the literature, it is difficult to determine the direction of changes in egg weight with advancing age of Japanese quail females. This trait is likely to be affected by other environmental and nutritional factors that might be partially confounded with age. Nhan *et al.* (2018) observed an increase in the contribution of eggshell to egg weight in eggs laid by quail aged 10 to 26 weeks, with this proportion again decreasing significantly afterwards.

The chemical composition of eggs changed with age of Japanese quail hens (Table 2). The least water was found in eggs from the oldest birds. The difference between this group and 15-week-old quail amounted to 1.24 percentage points (P <0.05). The percentage of crude fat content increased with the age of birds (P <0.05) and the percentage of crude protein was also found in eggs from the oldest quail. Likewise, the crude ash content in eggs from 15-week-old hens was less than in the eggs from older birds (P <0.05). The yolks and albumin from eggs produced by 31-week-old quail were more acidic than those in eggs produced by younger birds.

Table 2 Effect of age of Japanese quail on the chemical composition of egg yolk and egg white

Trait	15th week	23rd week	31st week	Overall
Water, %	73.07^{a} ± 0.13	71.98^{b} ± 0.46	70.43^{c} ± 0.33	71.83 ± 0.31
Crude protein, %	13.09^{b} ± 0.13	12.94^{b} ± 0.14	13.58^{a} ± 0.19	13.20 ± 0.15
Crude fat, %	11.58^{c} ± 0.28	12.65^{b} ± 0.33	13.44^{a} ± 0.34	12.56 ± 0.32
Crude ash, %	1.24^{b} ± 0.02	1.43^{a} ± 0.09	1.55^{a} ± 0.05	1.41 ± 0.05
pH of yolk	5.78^{a} ± 0.07	5.79^{a} ± 0.15	5.33^{b} ± 0.02	5.63 ± 0.08
pH of white	8.70^{a} ± 0.02	8.59^{a} ± 0.10	8.30^{b} ± 0.02	8.53 ± 0.05

^{a,b} Within a row, means with a common superscript did not differ with probability *P* =0.05

Dudusola (2010) reported chemical characteristics of Japanese quail eggs similar to the present results. However, Genchev (2012) reported a lower crude ash content in the eggs of these birds ($\bar{x}$ = 0.89-0.98%) than was observed here. It is difficult to unequivocally explain the changes in the basic chemical composition of eggs with the age of Japanese quail, especially since there were no significant changes in egg weight in the present study where the diet and environmental conditions were consistent over time. Perhaps, as the quail ages, the ability to deposit water, protein, fat, etc., in the eggs of these birds changes. Using multiple hybrids in two periods of laying, Czaja and Gornowicz (2006) observed similar relationships to those in the present study. These authors noted a higher total protein content in egg white and egg yolk from older hens and a higher water content in egg white of younger females. The least variable traits of eggs from different laying periods of the hens that were studied by Czaja and Gornowicz (2006) were the ash contents in both egg white and yolk.

Teuşan *et al.* (2009) found slightly higher pH values for yolk and white (6.07 ± 0.062 and 8.90 ± 0.014, respectively) than those that were seen in this research. Different results were obtained by Silversides and Scott (2001) and Akyurek and Okur (2009), who observed increased pH of the egg white with age of the hen. According to Węsierska (2006), the alkaline pH of the egg white inhibits the growth of microorganisms in eggs significantly. Therefore, the increase in pH of the egg white in eggs from older birds – which are characterised by inferior eggshell quality that might lead to a greater possibility of migration of microorganisms into the egg – seems to be a logical defence mechanism. The best condition for development of most bacterial species is a neutral pH, and pH in the range of 2 to 6 is deemed optimal for microscopic fungi. Although a significant decrease in pH of the egg white was observed with the advancing age of the hens in the present study, the pH remained at an appropriate level to provide an antibacterial defence (Guyot *et al.*, 2016).

Eggs laid by 23- and 31-week-old birds were characterized by higher contents of C14:0, C18:1, C18:2, C18:3, and C20:0 and lower contents of C20:1, C20:2, C20:3, and C20:4 compared with 15-week-old females. Eggs from the 31-week-old quail had higher proportions of C17:0 and C17:1 in the yolk compared with younger birds. Thus, eggs produced by 23- and 31-week-old hens had significantly higher PUFA and lower SFA contents in comparison with the youngest birds. The lowest cholesterol content was found in egg yolks from 23-week-old quail. The youngest and oldest birds had similar values of this sterol in 1 g of yolk lipid and the whole egg.

Table 3 Effect of age on fatty acid (%) and cholesterol contents of egg yolk from Japanese quail

Trait	15th week	23rd week	31st week	Overall
Myristic acid (C14:0)	$0.55^{b} \pm 0.01$	$0.66^{a} \pm 0.01$	$0.70^{a} \pm 0.03$	0.64 ± 0.02
Palmitic acid (C16:0)	$28.05^{a} \pm 0.23$	$26.13^{b} \pm 0.22$	$25.94^{b} \pm 0.41$	26.71 ± 0.29
Palmitoleic acid (C16:1)	$6.76^{a} \pm 0.24$	$5.70^{b} \pm 0.17$	$6.02^{ab} \pm 0.35$	6.16 ± 0.25
Margaric acid (C17:0)	$0.15^{b} \pm 0.01$	$0.16^{ab} \pm 0.01$	$0.18^{a} \pm 0.01$	0.16 ± 0.01
Heptadecenoic acid (C17:1)	$0.15^{b} \pm 0.01$	$0.15^{b} \pm 0.01$	$0.18^{a} \pm 0.01$	0.16 ± 0.01
Stearic acid (C18:0)	$8.81^{a} \pm 0.32$	$7.53^{b} \pm 0.46$	$7.29^{b} \pm 0.58$	7.88 ± 0.45
Oleic acid (C18:1)	$43.74^{b} \pm 0.63$	$46.18^{a} \pm 0.21$	$46.61^{a} \pm 0.79$	45.51 ± 0.54
Linoleic acid (C18:2)	$8.86^{b} \pm 0.42$	$11.22^{a} \pm 0.15$	$11.15^{a} \pm 0.41$	10.41 ± 0.33
Linolenic acid (C18:3)	$0.14^{c} \pm 0.01$	$0.26^{b} \pm 0.01$	$0.40^{a} \pm 0.03$	0.27 ± 0.02
Arachidic acid (C20:0)	$0.04^{c} \pm 0.00$	$0.15^{a} \pm 0.02$	$0.06^{b} \pm 0.01$	0.08 ± 0.01
Gadoleic acid (C20:1)	$0.23^{a} \pm 0.01$	$0.17^{b} \pm 0.00$	$0.18^{b} \pm 0.01$	0.19 ± 0.01
Eicosadienoic acid (C20:2)	$0.25^{a} \pm 0.01$	$0.07^{c} \pm 0.01$	$0.11^{b} \pm 0.01$	0.14 ± 0.01
Mead acid (C20:3)	$0.18^{a} \pm 0.01$	$0.08^{b} \pm 0.01$	$0.11^{b} \pm 0.01$	0.12 ± 0.01
Arachidonic acid (C20:4)	$1.82^{a} \pm 0.11$	$1.15^{b} \pm 0.09$	$0.98^{b} \pm 0.21$	1.32 ± 0.14
Monounsaturated fatty acids	50.99 ± 0.83	52.39 ± 0.25	52.98 ± 0.99	52.02 ± 0.69
Polyunsaturated fatty acids	$11.35^{b} \pm 0.56$	$12.87^{a} \pm 0.63$	$12.79^{a} \pm 0.59$	12.26 ± 0.46
Satuated fatty acids	$37.66^{a} \pm 0.41$	$34.74^{b} \pm 0.35$	$34.23^{b} \pm 0.64$	35.47 ± 0.40
Cholesterol, mg/egg	$72.14^{a} \pm 1.76$	$50.60^{b} \pm 4.29$	$66.49^{a} \pm 3.60$	63.10 ± 3.22
Cholesterol, mg/g lipid	$50.95^{a} \pm 2.38$	$36.03^{b} \pm 2.20$	$48.26^{a} \pm 2.38$	50.16 ± 2.06

^{a,b} Within a row, means with a common superscript did not differ with probability P =0.05

Monounsaturated fatty acids had the largest share of the lipid profile in this study. This was consistent with the results of Choi *et al.* (2001) and Polat *et al.* (2013) but differs from those of Sokołowicz *et al.* (2012). Although it appears that nutrition has a primary influence on the fatty acid profile of egg yolk, Cherian (2008) observed that the effect of the age of broiler breeder hens was significant. Calik (2016) found that increases in the age of laying hens raised the level of cholesterol in their eggs. Zgłobica *et al. (*1995) attributed the increase in cholesterol content in yolks with increased age of laying hens to a decrease in the rate of egg production. Similar trends were observed by Krawczyk (2009). Additionally, negative relationships between the cholesterol content in the yolk, its weight, and the relative contribution of yolk weight to egg mass were shown by Stępińska *et al. (*1996). However, the results of the curent study did not confirm these relationships because there were no differences in the weight or relative weight egg yolk from quail that differed in age.

The oldest birds produced eggs that had significantly greater concentrations of zinc, selenium, copper and manganese in the edible portion (yolk and white) of the egg (Table 4). The edible portion of eggs produced by 23-week-old birds was characterized by greater concentrations of magnesium and iron compared with the eggs from hens that were older or younger.

Table 4 Effect of age of Japanese quail on macro- and microelement composition in egg yolk and white

Trait, mg/kg	15th week	23rd week	31st week	Overall
Calcium	592.1 ± 0.29	625.0 ± 0.45	623.7 ± 0.74	613.6 ± 0.49
Magnesium	161.2^{c} ± 2.73	181.1^{a} ± 6.63	173.6^{b} ± 4.92	171.9 ± 4.76
Iron	48.10^{b} ± 1.86	52.45^{a} ± 0.76	48.05^{b} ± 1.76	49.53 ± 1.46
Zinc	14.67^{b} ± 0.13	15.86^{a} ± 0.39	16.46^{a} ± 0.20	15.66 ± 0.24
Selenium	0.327^{b} ± 0.01	0.287^{c} ± 0.01	0.407^{a} ± 0.01	0.340 ± 0.01
Copper	0.279^{b} ± 0.02	0.233^{b} ± 0.03	0.517^{a} ± 0.02	0.343 ± 0.02
Manganese	0.015^{b} ± 0.00	0.014^{b} ± 0.00	0.018^{a} ± 0.00	0.016 ± 0.00
Cadmium	0.357 ± 0.04	0.481 ± 0.06	0.362 ± 0.03	0.400 ± 0.04
Nickel	0.477^{a} ± 0.05	0.491^{a} ± 0.05	0.315^{b} ± 0.02	0.428 ± 0.04
Cobalt	0.523^{a} ± 0.02	0.393^{b} ± 0.03	0.475^{a} ± 0.02	0.464 ± 0.02
Lead	0.139^{b} ± 0.01	0.157^{a} ± 0.00	0.146^{ab} ± 0.01	0.147 ± 0.01

^{a,b} Within a row, means with a common superscript did not differ with probability P =0.05

The contents of Fe, Mn, Zn and Cu in the edible portion of quail eggs in this study was consistent with results from Tunsaringkarn *et al. (*2013). Similar to fatty acids, the levels of macro- and microelements in eggs depend to a large extent on nutrition. However, some authors indicated that the housing system may have an effect on the mineral content of eggs (Drabik *et al.,* 2018). The lack of studies on changes in the level of elements in eggs with the age of females does not allow far-reaching conclusions to be drawn from the present research. Moreover, the proportion of certain elements increased with the age of quail and others decreased. Such irregular changes may be associated with the different uses of these components by the female and their uneven deposition in eggs.

As the hens became older, the content and activity of lysozyme in the protein decreased (Table 5). Eggs collected from 15- and 23-week-old birds had a similar lysozyme contents, amounting to approximately 0.228%. Hydrolytic activity of lysozyme in egg white from eggs produced by 23- and 31-week-old hens was lower than for 15-week-old hens.

Table 5 Effect of Japanese quail age on lysozyme level in egg white

Trait	15th week	23rd week	31st week	Overall
Content in albumen liquid (%)	0.232^{a} ± 0,01	0.223^{a} ± 0,01	0.191^{b} ± 0,01	0.215 ± 0,01
Activity (U/ml)	41223^{a} ± 195,42	38915^{b} ± 290,71	34161^{c} ± 345,10	38099 ± 277,10

^{a,b}Within a row, means with a common superscript did not differ with probability P =0.05

The content of lysozyme in bird eggs, as an antimicrobial protein, may be highly variable and depends on many factors (Trziszka *et al.*, 2007; Fang *et al.*, 2012; Nowaczewski *et al.*, 2013), the age of the females being one of them. In domestic geese, a significantly lower content and activity of lysozyme was found only in eggs from four-year-old birds (Adamski *et al.*, 2016). Similarly, Banaszewska *et al. (*2019) showed more activity of lysozyme in older broiler breeders (60 weeks old) compared with birds at 30 weeks old. This might be because eggs from older females are usually of inferior quality (Kontecka *et al.*, 2012), primarily because the eggshell becomes thinner and less durable, resulting in greater ability of pathogenic microorganisms to penetrate the egg. Therefore, a higher content or activity of lysozyme in egg white is a form of antibacterial defence mechanism when the first protective barrier (the eggshell) deteriorates with the bird's age. However, the results of the research did not confirm this hypothesis, because the content and activity of lysozyme in egg white decreased with the age of Japanese quail. Attempts to explain this phenomenon were undertaken by Ahlborn and Sheldon (2005) in studies on hens. The authors hypothesized that perhaps as egg production decreases, other hormonal and physiological changes occur in the hen, such as a reduction in lysozyme in the oviduct, albumen, and shell membrane.

Conclusions

Effects were not found of age of Japanese quail on the physical characterization their eggs. From the point of view of consumers, eggs from older birds appear to be more valuable because they have more PUFAs and crude protein, and a higher concentration of minerals. However, antibacterial properties of the eggs deteriorate as the quail ages, which may indicate a shorter shelf life.

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Authors' Contributions

SN conceived the research, designed the experiment, analysed the data and drafted the article; TS, RC-R, KS-Sz, MR, ŁT and KSz conducted and interpreted the biochemical analyses; KP collected the data and prepared it for statistical evaluation; SK and MH revised the manuscript, including first language correction and final review.

Conflict of Interest Declaration

The authors have no conflicts of interest relative to this research.

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