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EFFECT OF FEED SUPPLEMENTATION WITH ORGANIC SELENIUM AND VITAMIN E ON CHEMICAL COMPOSITION AND SENSORY CHARACTERISTICS OF JAPANESE QUAIL (*COTURNIX JAPONICA*) EGGS

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Key words: Japanese quail, egg chemical composition, selenium, vitamin E

The objective of this study was to evaluate the chemical composition and sensory characteristics of eggs collected in three periods of the laying cycle from quails fed with feeds supplemented by organic selenium and vitamin E.

Quail females were randomly divided into two groups (48 birds each): control (CG) and experimental (EG). From the first day of live to the end of laying cycle birds of the CG were fed the basic feeds, appropriate to age, while of the EG, with feeds supplemented with 0.3 mg/kg selenium (as 300 mg of selenium yeast – Sel-Plex®, Alltech LTD, USA) and 100 mg/kg vitamin E (200 mg/kg of E-50 Adsorbate – Rolimpex S.A). From each group 20 randomly selected eggs were collected at the beginning (1st month of production), peak (4th month) and from the end of the laying cycle (10th month) and subjected to analysis. The following traits were evaluated: contents of albumen, fat, ash and dry matter in relation to egg weight; contents of selenium, cholesterol, albumen and retinol in the yolk; lysozyme and cystatin activity in the egg white. Moreover, the eggs were subjected to sensory evaluation in five-grade consumer scale: overall appearance, taste, flavour, consistency of egg white and yolk, and yolk colour were examined.

Chemical analysis of egg essence revealed that fat content in the eggs collected from the CG was, at the peak (11.5%) and entire laying period (11.4%) significantly ($p \leq 0.05$) higher than from the EG (10.8% and 10.7%, respectively). Differences in percentage of albumen, minerals and dry matters were not significant.

Feed supplementation with organic selenium and vitamin E increased significantly ($p \leq 0.05$) selenium content in egg yolk both, collected at the peak and the end of laying cycle. Average value for entire period amounted 0.47 g/10 mL in the EG and 0.37 g/10 mL in the CG. Also albumen content in the eggs of EG (average for entire cycle – 96.4 mg/mL) was higher ($p \leq 0.05$) than in the CG (92.2 mg/mL). Feed supplementation with organic selenium and vitamin E increased significantly ($p \leq 0.05$) cholesterol level in the yolk. For the entire laying cycle it averaged 13.1 mg/g in EG and 11.8 mg/g in CG.

Activity of biological substances in the egg white was changing in the analyzed periods of the laying cycle. At the beginning of cycle the lysozyme activity in the eggs of the EG was higher ($p \leq 0.05$), while cystatin activity was lower ($p \leq 0.05$) when compare with the CG. However, at the peak of laying lysozyme activity in the egg white of CG increased ($p \leq 0.05$) while in eggs of EG decreased, therefore for the entire laying cycle existing difference was not significant. Similar variations were observed in relation to cystatin. At the beginning and peak of cycle its activity in CG (3.4 U/5 mg and 3.3 U/5 mg) was higher ($p \leq 0.05$) than in EG (3.0 U/5 mg and 2.7 U/5 mg, respectively) while at the end was opposite (3.3 U/5 mg in EG and 3.0 U/5 mg in CG; $p \leq 0.05$). For all evaluated periods the taste, flavour, consistency of egg white and yolk, and yolk colour of eggs from CG were higher ($p \leq 0.05$) comparing to EG.

INTRODUCTION

Selenium is an element necessary for normal body function in humans and many species of animals. Recommended daily allowance for selenium is 60 μg/day for women and 70 μg/day for men [Marzec *et al*., 2002; Sheng *et al*., 2002]. Marzec *et al*. [2002], who determined the structure of selenium intake from different groups of food products in Poland, found that the main source of selenium are meat and meat products (27.2%), whereas the intake of this element from eggs is only 9.1%.

Feeding layers a diet supplemented with organic selenium increases the concentration of this element in eggs [Dobrzański *et al*., 2001; Payne *et al*., 2005; Surai *et al*., 2006], which makes the eggs a source of easily available selenium in human diets.

Like vitamin E, selenium is necessary for the normal course of the cellular and humoral immune response [Furowicz *et al*., 1993; Sahin *et al*., 2002]. The content and activity of biologically-active substances in eggs may increase through the stimulation of the immune system in females [Sim *et al*., 2000]. Egg white is rich in biologically active proteins such as lysozyme (which has antiviral and bacteriostatic properties) and cystatin (which has antiviral properties) [Kopeć *et al*., 2005]. Thanks to these properties, lysozyme is commonly used in food production as a bioprotective agent and in the cosmetic industry as a natural antibiotic [Cunningham *et al*., 1991].

Feeding animals diets with elevated selenium level is closely related to vitamin E. Vitamin E has been found to increase the body's requirement for selenium [Furowicz *et al*., 1993].

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Considering the importance of selenium and vitamin E in the human diet, a study has been carried out to determine the effect of adding organic selenium and vitamin E to diets on the chemical composition and sensory properties of Japanese quail eggs, evaluated over the entire egg production cycle.

MATERIALS AND METHODS

Freshly hatched quails were randomly divided into two groups: the control group (CG) fed, from the first day of live to the end of the laying cycle, with the basic feeds (appropriate to birds age) and the experimental group (EG) fed with feeds supplemented with 0.3 mg/kg selenium (as 300 mg of selenium yeast – Sel-Plex®, Alltech LTD, USA) and 100 mg/kg vitamin E (200 mg/kg of E-50 Adsorbate – Rolimpex S.A).

From sexually matured flocks (at 7th week of age) 48 females were selected for further experiment and placed into two 4-tier battery cages (1.0 x 0.55 x 0.3 m of each level and for 12 birds). All cages were kept indoor at temperature 22--24°C. During the laying birds were treated with 14 h light/10 h dark cycle. Water and feed were available *ad libitum*.

Egg analysis. The eggs collected at the beginning (1st month of production), peak (4th month) and at the end of laying cycle (10th month) were evaluated at the day of collection.

In 20 eggs (with average weight of 9.5 g) collected from each group and laying period there were evaluated individually: contents of protein according to Kjeldahl method [PN-75/A-04018], fat content with Soxhlet method [PN ISO 1444:2000], ash [PN-ISO 936:2000] and dry matter [PN-A-86509:1994] in relation to egg weight. Selenium content was analysed in the whole egg mass by mineralization procedure using microwave oven, then pressure organic sample digestion with concentrated, spectrally pure, nitric acid in microprocessor microwave station MDS-2000 (CEM, USA). Chemical analysis of Se content was carried out with the use of ICP-MS technique from Varian, Ultra Mass 700 [Górecka & Górecki, 2000]. Egg yolk was treated with methylene chloride and methanol mixture (2:1) [Folch *et al.,* 1957] in order to extract lipids for further procedures. Cholesterol content was analysed using a liquid chromatographic method (Agilent 1100 Series) in a column XDB-C18. Vitamin A (retinol) concentration was determined with high pressure liquid chromatography at 292 nm. Egg albumen was proceeded to lysozyme and cystatin activity measurements. Specific activity of lysozyme was determined by spectrophotometric (turbidimetric) method at a wavelength of 450 nm. The change in the suspension of *Micrococcus lysodeiticus* bacteria absorbance was registered during the reaction of enzyme with the bacterial cells for 6 min. Activity of cystatin was determined as the ability to inhibit enzymatic activity of papain using BANA (N-benzoil-DL arginyl- β-naphtylamide hydrochloride) as a substrate after incubation of the samples at 37oC. The reaction was stopped by the addition of DMBA (p-dimethyloaminobenzaldehyde) and decrease of the absorbance was measured spectrophotometrically at a wavelength of 450 nm [Nishida *et al*., 1984; Siewiński, 1991]. Moreover, boiled eggs were subjected to sensory evaluation in a five-grade consumer scale of acceptance, where 1 = not acceptable; 2 = moderately acceptable; 3 = slightly acceptable; 4 = acceptable; 5 = very acceptable [PN-ISO 4121:1998]. Consumers were asked to evaluate the overall appearance, taste, flavour, yolk colour, consistency of egg white and yolk.

Obtained data were analysed statistically with ANOVA, the significance of differences by Duncan's multiple range test (Statistica 7.1).

The presented experiment was approved by the II Local Ethics Commission for Experiments Carried on Animals.

RESULTS AND DISCUSSION

Chemical analysis of egg content showed that eggs from the control group (CG) had a significantly ($p \leq 0.05$) higher proportion of fat at the peak of egg production (11.5%) and during the entire egg production cycle (11.4%) compared to that found in eggs from the experimental group (EG) (10.8% and 10.7%, respectively) (Table 1). It can be concluded that feeding Japanese quail a diet enriched with Se and vitamin E reduces the proportion of fat, because the result obtained in the CG group (11.49%) is comparable to that reported by Szczerbińska *et al*. [1998]. The differences found in the proportion of protein, mineral substances and dry matter in the analyzed groups and laying periods were not significant (Table 1), but in both groups the percentage of protein and dry matter was higher throughout the egg production period compared to that reported by Szczerbińska *et al*. [1998], by 2.51 and 1.88 percentage points (PP), respectively.

At present, one of the strategies used in the agri-food industry is to produce food enriched with elements and other substances promoting human health and beauty. One of these is selenium. Feeding birds diets supplemented with organic Se enables the production of Se-enriched eggs [Surai *et al*., 2004, 2006; Kardas *et al*., 2004; Golubkina & Papazyan, 2006], meat and livers [Łukaszewicz *et al*., 2007a,b]. According to Surai *et al*. [2004], where selenium is deficient in the human diet, the daily intake of two Se-enriched eggs for eight weeks significantly increases the concentration of Se in blood. Compared to CG egg yolk, the Se content of egg yolk from the EG group was higher ($p \leq 0.05$) both, at the peak and at the end of the egg production cycle, with a 1.3-fold increase in Se concentration found in the entire egg production cycle. Kardas *et al*. [2004] and Surai *et al*. [2006] found a 1.8-fold increase in Se concentration in yolk after feeding Japanese quail a diet containing 0.5 mg/kg Se for six months.

In the analysed egg production periods, EG eggs were found to be higher ($p \leq 0.05$) in yolk protein compared to CG eggs. Mean values for the whole cycle were 96.4 mg/ml for EG eggs and 92.2 mg/ml for CG eggs (Table 1). The Se and vitamin E supplement significantly ($p \leq 0.05$) reduced the level of yolk cholesterol, which averaged 11.8 mg/g in the EG group and 13.1 mg/g in the CG group (Table 1) for the entire egg production cycle. The results obtained in the EG were lower than those reported by Bragagnolo & Rodriguez-Amaya [2003] (12.1 mg/g yolk) and Szczerbińska *et al*. [1998] (15.13 mg/g), and higher than those found by Kaźmierska *et al*. [2005] (7.78 mg/g yolk).

The activity of active substances in egg white varied according to the egg production period analysed. In the initial period, lysozyme activity in EG eggs was higher ($p \leq 0.05$), and that of

TABLE 1. The effect of organic selenium and vitamin E addition on chemical composition of quails (*Coturnix japonica*) eggs collected during the entire laying cycle (means±SD).

Period of the laying cycle	Group	Egg chemical composition (%)				Contents in the yolk				Activity in egg white	
		Albumen	Fat	Ash	Dry matter	Selenium (g/10 mL)	Cholesterol (mg/g)	Retinol (μg/egg)	Albumen (mg/mL)	Lysozyme (U/5 mg)	Cystatin (U/5 mg)
Beginning	Experimental	12.3±1.10	10.8±1.30	0.8±0.10	26.1±0.20	0.37±0.01	$11.9^{a}\pm0.02$	10.0±0.01	$100.2^{a}\pm3.67$	$136.7^{a}\pm7.66$	$3.0^{a}\pm0.14$
	Control	12.5±1.20	11.0±1.50	0.8±0.10	26.0±0.20	0.37±0.01	$14.1^{b}\pm0.02$	10.1±0.04	$95.9^{b}\pm1.49$	$115.8^{b}\pm6.98$	$3.4^{b}\pm0.19$
Peak	Experimental	12.9±1.30	$10.8^{a}\pm1.30$	0.8±0.10	26.4±0.20	$0.48^{a}\pm0.01$	$12.1^{a}\pm0.02$	9.8±0.03	$100.0^{a}\pm3.65$	$119.1^{a}\pm5.60$	$2.7^{a}\pm0.10$
	Control	12.8±1.10	$11.5^{b}\pm1.50$	0.8±0.10	26.2±0.20	$0.37^{b}\pm0.01$	$12.3^{b}\pm0.01$	9.8±0.02	$96.9^{b}\pm1.33$	$130.1^{b}\pm8.34$	$3.3^{b}\pm0.21$
End	Experimental	13.1±1.20	11.0±1.50	0.8±0.10	26.5±0.30	$0.56^{a}\pm0.03$	$11.4^{a}\pm0.02$	9.8±0.02	$88.9^{a}\pm2.60$	$130.1^{a}\pm11.25$	$3.3^{a}\pm0.12$
	Control	12.9±1.21	11.8±1.42	0.8±0.10	26.4±0.20	$0.37^{b}\pm0.01$	$12.8^{b}\pm0.02$	9.7±0.02	$83.8^{b}\pm2.19$	$142.5^{b}\pm8.27$	$3.0^{b}\pm0.20$
Total	Experimental	12.8±0.10	$10.7^{a}\pm0.12$	0.8±0.10	26.3±0.21	$0.47^{a}\pm0.08$	$11.8^{a}\pm0.37$	9.9±0.10	$96.4^{a}\pm0.42$	128.6±23.03	$3.0^{a}\pm0.54$
	Control	12.7±0.15	$11.4^{b}\pm0.40$	0.8±0.10	26.2±0.20	$0.37^{b}\pm0.01$	$13.1^{b}\pm0.95$	9.8±0.15	$92.2^{b}\pm0.21$	129.4±20.53	$3.2^{b}\pm0.47$

Values in columns with different letters within laying period differ significantly (a, b – $p\leq0.05$)

TABLE 2. The effect of organic selenium and vitamin E addition on sensory characteristics of quails (*Coturnix japonica*) eggs collected during the entire laying cycle (means±SD).

Period of the laying cycle	Group	Taste	Flavour	Overall appearance	Consistency of:		Colour
					Yolk	Egg white	
Beginning	Experimental	$4.3^{a}\pm0.27$	$3.9^{a}\pm0.33$	4.2±0.35	$4.3^{a}\pm0.20$	$4.6^{a}\pm0.19$	$4.2^{a}\pm0.28$
	Control	$4.6^{b}\pm0.30$	$4.5^{b}\pm0.28$	4.8±0.25	$4.9^{b}\pm0.40$	$4.9^{b}\pm0.33$	$4.8^{b}\pm0.25$
Peak	Experimental	$4.3^{a}\pm0.24$	$4.1^{a}\pm0.32$	4.2±0.25	$4.5^{a}\pm0.23$	$4.6^{a}\pm0.23$	$4.4^{a}\pm0.29$
	Control	$4.7^{b}\pm0.32$	$4.5^{b}\pm0.30$	4.8±0.24	$4.8^{b}\pm0.40$	$4.9^{b}\pm0.37$	$4.8^{b}\pm0.24$
End	Experimental	$4.4^{a}\pm0.29$	$4.1^{a}\pm0.32$	4.3±0.30	$4.4^{a}\pm0.20$	$4.5^{a}\pm0.20$	$4.4^{a}\pm0.31$
	Control	$4.7^{b}\pm0.25$	$4.5^{b}\pm0.28$	4.8±0.25	$4.9^{b}\pm0.42$	$4.9^{b}\pm0.30$	$4.8^{b}\pm0.24$
Total	Experimental	$4.3^{a}\pm0.76$	$4.1^{a}\pm0.73$	4.2±0.77	$4.4^{a}\pm0.77$	$4.6^{a}\pm0.77$	$4.4^{a}\pm0.77$
	Control	$4.7^{b}\pm0.66$	$4.5^{b}\pm0.63$	4.8±0.65	$4.9^{b}\pm0.66$	$4.9^{b}\pm0.66$	$4.8^{b}\pm0.65$

Values in columns with different letters within laying period differ significantly (a, b – $p\leq0.05$)

cystatin lower, in relation to CG eggs. However, at the peak of egg production, the activity of lysozyme in the white of CG eggs increased and that in the white of EG eggs decreased, resulting in non-significant differences for the whole egg production cycle (128.6 U/5 mg of white in the EG group and 129.4 U/5 mg of white in the CG group). Cystatin activity showed similar variation. In the initial period of the cycle, cystatin activity in the CG group (3.4 and 3.3 U/5 mg of white) was higher ($p \leq 0.05$) than in the EG group (3.0 and 2.7 U/5 mg of white), but in the final period these values were reversed (3.3 U/5 mg of white in the EG group and 3.0 U/5 mg of white in the CG group). In the entire egg production cycle, cystatin activity in the CG group (3.2 U/5 mg of white) was higher ($p \leq 0.05$) in relation to cystatin activity in the EG group (3.0 U/5 mg of white) (Table 1). It is therefore concluded that feeding Japanese quail diets supplemented with antioxidants did not increase the activity of the biologically active substances analysed in the eggs.

The biological activity of lysozyme and cystatin in the Japanese quail eggs analysed is considerably lower than in the egg white of geese or hens. Chrzanowska *et al*. [2005] found the activity of lysozyme to be 204.4 U/5 mg of white and the activity of cystatin to be 36.9 U/5 mg of white in the eggs of White Italian geese, compared to 7415 U/5 mg of white and 12.10 U/5 mg of white in hen eggs [Kopeć *et al*., 2005]. When feeding hens a diet containing a 3% supplement of fish-mineral concentrate with humocarbovit and antioxidants (vitamins E and A), Kopeć *et al*. [2005] found that in relation to the control group, lysozyme activity decreased by 663 U/5 mg of white and cystatin activity decreased by 2.42 U/5 mg of white.

The sensory evaluation of eggs showed that the eggs of Japanese quail fed selenium and vitamin E diets were less attractive. In all the periods of the egg production cycle analysed, the flavour, aroma, yolk and white consistency and yolk colour were significantly ($p \leq 0.05$) better in CG eggs compared to EG eggs (Table 2). However, the results of sensory evaluation of eggs in other countries possibly varied due to different consumer preferences, as reported by Roberts [2004]. Although there were significant differences in individual sensory traits of eggs, in both groups they exceeded 4 points, which makes them highly attractive.

Nevertheless, the reduced desirability of EG eggs is compensated by increased Se concentration in yolk, thanks to which these eggs are considered Se-enriched quail eggs, which is important in countries where Se intake is too low.

CONCLUSIONS

1. Feeding Japanese quail a diet enriched with selenium and vitamin E reduces the concentration of fat in eggs and the concentration of cholesterol in yolk, while increasing the levels of selenium and protein.

2. Adding antioxidants to the diet does not increase the level of biologically active substances in the egg white of Japanese quail.

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WPŁYW DODATKU SELENU ORGANICZNEGO I WITAMINY E NA SKŁAD CHEMICZNY ORAZ CECHY SENSORYCZNE JAJ PRZEPIÓREK JAPOŃSKICH (*COTURNIX JAPONICA*)

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Celem badań była ocena wpływu dodatku do paszy selenu organicznego i witaminy E na skład chemiczny oraz cechy sensoryczne jaj przepiórczych ocenianych na przestrzeni całego cyklu nieśnego.

Od pierwszego dnia życia ptaki z grupy kontrolnej (CG) żywiono paszami podstawowymi, a ptakom z grupy doświadczalnej (EG) dodawano do paszy selen organiczny w ilości 0,3 mg na 1 kg paszy (w postaci drożdży selenowych Sel-Plex®, Alltech LTD, USA) i witaminę E (E-50 Adsorbate, Rolimpex S.A.) w ilości 100 mg na 1 kg paszy.

Analizie poddano jaja jednodniowe zbierane w trzech okresach cyklu nieśnego: początkowym, szczycie oraz w końcowym. Określano procentowy udział w treści jaja: białka, tłuszczu, substancji mineralnych i suchej masy; zawartość selenu, cholesterolu, białka i retinolu w żółtku, a także aktywność lizozymu i cystatyny w białku. Ponadto jaja poddano 5. punktowej ocenie cech sensorycznych określając wygląd ogólny, smak, zapach, konsystencję żółtka i białka oraz barwę żółtka.

Analiza chemiczna treści jaj wykazała, że udział tłuszczu w jajach z grupy kontrolnej (CG) był istotnie ($p \leq 0,05$) wyższy (11,4%) w stosunku do stwierdzonego (10,7%) w jajach z grupy doświadczalnej (EG).

Zawartość selenu w żółtkach jaj EG była wyższa ($p \leq 0,05$) w porównaniu do CG i wynosiła odpowiednio: 0,47 g/10 mL i 0,37 g/10 mL. Również zawartość białka w żółtku jaj z EG była wyższa niż w grupie CG i w cały cyklu wynosiła średnio 96,4 mg/mL w EG i 92,2 mg/mL w CG. Dodatek selenu i witaminy E istotnie ($p \leq 0,05$) obniżył poziom cholesterolu w żółtku, który wynosił średnio dla całego cyklu nieśności 11,8 mg/g w EG i 13,1 mg/g w CG. Aktywność lizozymu między badanymi grupami była nieistotna (128,6 U/5mg w EG i 129,4 U/5mg w CG), natomiast aktywność cystatyny w CG była wyższa ($p \leq 0,05$) wynosząc 3,2 U/5mg w porównaniu do 3,0 U/5mg w EG. We wszystkich analizowanych okresach cyklu nieśnego smak, zapach, konsystencja żółtka i białka oraz barwa żółtka jaj z CG były istotnie ($p \leq 0,05$) wyższe w porównaniu do jaj z EG.