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EFFECT OF DIET SUPPLEMENTED WITH BLACK CUMIN (*NIGELLA SATIVA*) SEEDS ON EGG YOLK FATTY ACID PROFILE AND EGG YOLK CHOLESTEROL LEVEL OF JAPANESE QUAIL (*COTURNIX JAPONICA*)

Danuta Szczerbińska, Marta Sulik, Danuta Majewska✉, Marek Ligocki, Michalina Adaszyńska-Skwirzyńska

Department of Monogastric Animal Sciences, West Pomeranian University of Technology in Szczecin, Klemensa Janickiego 29, 71-460 Szczecin, Poland

ABSTRACT

The aim of the study was to determine whether a diet with an addition of black cumin seeds (BCS) can be used in functional egg production. At 8 weeks of age, a flock of 96 female quails was divided into 3 groups. The control group received their standard feed (0 BCS). The diet offered to the test groups was supplemented with BCS in the amount of 20 and 50 g · kg^{-1}, respectively. Dietary BCS supplementation decreased feed intake and improved feed efficiency. Egg yolk of the quail groups treated with BCS in their diet had by a lower percentage of SFA and an increased level of UFA. A significantly increase was observed in the level of PUFA, especially in PUFA n-6. The health-promoting properties of the product were indicated by the fact of a considerable reduction in the yolk cholesterol level. Unfortunately, n-6/n-3 ratio increased in the groups offered the BCS-supplemented diet and under these circumstances eggs obtained from quails receiving BCS in the diet did not meet the requirements of functional food.

Key words: black cumin seeds, Japanese quail, feeding, performance, functional egg

INTRODUCTION

The type of feed offered to hens – fat they contain in particular – has an effect on the amount of lipids contained in the eggs. This chiefly refers to some fatty acids that positively affect the human cardiovascular system through lowering the triglyceride level and increasing the HDL cholesterol level in blood. Modification of the chemical composition of eggs using appropriately composed diets aims at reaching a product with the nutraceutical character, which would be a more beneficial form of the everyday diet compared to direct consumption of plant or fish oils [Milinsk et al. 2003, Carrillo-Domìnguez et al. 2005, Atakisi et al. 2009]. One of the plant products with a high content of components important from a nutritional point of view is black cumin (*Nigella sativa* L). It has been confirmed that its seeds contain a substantial quantity of minerals and vitamins, and are rich in valuable lipids [Takruri and Dameh 1998, Atta 2003], particularly UFA (82.0%), out of which 60.1% are polyenoic fatty acids and 21.9% are monoenic fatty acids [Yalçin et al. 2009]. Several therapeutic effects on human health have been shown in studies on black cumin seeds application. They find application in medicine as, among others, antihypertensive, gastrointestinal function regulators, diuretics or painkillers [Awad 2005, Rooney and Ryan 2005, Salem 2005, Gali-Muhtasib et al. 2006].

Consequently, the hypotheses were proposed that introducing BCS to the diet of birds would modify egg chemical composition so that the level of polyunsaturated fatty acids would increase, and cholesterol levels would be reduced in the yolk. It was also assumed that the diet composed this way should not deteriorate the performance of the birds.

✉ danuta.majewska@zut.edu.pl

Studies on the possibility of developing functional eggs through application of diets enriched with unconventional plant components have been mostly conducted on commercial laying hen cross-breeds [Atta 2003, El Bagir et al. 2006, Yalçin et al. 2009]. Few studies have utilized quails [Denli et al. 2004, Szczerbińska et al. 2012] which, due to their low living requirements and rapid growth and development, are a valuable research model. Moreover, quail eggs have excellent gustatory and dietetic qualities, while their high nutritional value also results from the fact that they can be included in the diet of people that are allergic to chicken egg albumen. Keeping in mind the growing demand for food showing health-promoting effects on humans, it was decided to evaluate the effect of BCS dietary supplementation on quail performance, egg yolk fatty acid profile and cholesterol level.

MATERIAL AND METHODS

Birds, diets and housing

All procedures were performed according to the guidelines for the care and use of research animals and were approved by the Local Ethics Committee on Animal Experimentation (West Pomeranian University of Technology in Szczecin, resolution number 14/2012, Szczecin, PL).

The study was carried out at an experimental farm of the Department of Poultry and Ornamental Birds, West Pomeranian University of Technology in Szczecin, Poland. The birds were Japanese quails (*Coturnix japonica*) raised in our own facilities. Quails were kept in group cages over the entire test period, with the housing conforming to the requirements of this species [Rutkowski 2000]. At age 8 weeks, the flock of 96 female quails, standardised by body weight, was divided into 3 groups with 4 replications, 8 birds each. The control group received the standard feed composed of corn meal, wheat meal and soybean post-extraction meal. The test diet was supplemented with BCS (*Nigella sativa* L.) in the amount of 20 and 50 g · kg^{-1} (Table 1). BCS were from crops cultivated in southern Poland. The composition of BCS and test diets (dry matter, crude protein, ether extract, crude fiber and crude ash) were determined by AOAC [Firestone 1990]. BCS fatty acids profiles were assayed using of gas chromatography.

Production parameters and egg quality characteristics

Over the entire test period, which lasted until week 23 of age, feed intake (FI), egg production (EP) and egg weight (EW), as well as deaths and culling, were recorded on a daily basis. These data were used to calculate basic production indices, i.e., average FI (g/quail/day), feed efficiency (FE) expressed as kg feed per kg eggs, EP (%) and EW (g).

Cholesterol content and yolk fatty acid profile

In the last week of the trial, 20 eggs from each group were randomly selected. Pooled samples were made of the separated yolks, out of which 12 samples were then collected from each group. Analyses of yolk lipids, involving total yolk cholesterol and fatty acids (FA) profile, were performed using gas chromatography. Preparation of the samples for total cholesterol level analysis (extraction, unsaponifiable matter separation, preparation of sterol trimethylsilyl ethers) and chromatographic analysis were carried out according to the Polish Standard PN-EN 12228 [Polska Norma 2002]. Yolk cholesterol was calculated and expressed as mg per g yolk.

Analyses of the FA levels were performed according to the Polish Standard PN-EN ISO 5508 [Polska Norma 1996]. The profile of FA was determined using gas chromatograph with mass detector (GC MS-CLARUS 600, prod. Perkin Elmer), using the ELITE-5MS capillary column – 60 m × 0.25 mm I.D. × 0.25 µm. The GC Parameters: Carrier gas: helium (He) 6.0, the flow of gas through the column at 1 ml/min; Dispensed volume: 1 ml; distribution of the sample in the dispenser (split) 50 : 1, injector temperature: 200°C Column temperature program: 110°C for 5 min, gradient of 5°C/min to 180°C, 180°C for 15 min gradient of 5°C/min to 290°C, 290°C for 5 min; transfer line temperature: 290°C. Standard was a mixture of 37 saturated and unsaturated fatty acids, prod. Supelco: FAME mix C4-C24 (cat. number: 18919–1AMP). Quantitative analysis was performed using the TURBOMAS software operating chromatograph and mass spectrometer, using peak area calculations. Extraction of the lipid compounds from egg yolk, their hydrolysis and transformation to the form of methyl esters were performed according to the Polish Standard PN-EN ISO 5509 [Polska Norma 2001]. The results were expressed as percentages of respective FA in the total content of all FA in a given sample. The data collected during this study were statistically processed with the Statistica ver. 10.0 package, using one-way ANOVA to examine differences among groups. Duncan's test [Duncan 1955] was used to test the differences between the control and experimental groups. The values were given as means ±standard deviations.

RESULTS AND DISCUSSION

Proximate analysis of BCS showed that seeds are rich in ether extract (Table 2). The results were similar to those reported by Atta [2003] and Yalçin et al. [2009]. Crude protein content was slightly higher than the results of the cited authors. A slight discrepancy in the protein

Table 1. Ingredients and chemical composition of diets (g · kg^{-1})

Tabela 1. Komponenty paszowe i skład chemiczny diet (g · kg^{-1})

Ingredients – Składniki	Dietary treatments, BCS supplementation Dieta z udziałem BCS		
Black cumin seeds meal – Mączka z nasion czarnuszki siewnej	0	20	50
Corn meal – Śruta kukrydziana	150	150	150
Wheat meal – Śruta pszenna	351.9	336.5	317.5
Barley meal – Śruta jęczmienna	160	160	160
Soybean oil – Olej sojowy	9	9	9
Post-extractive soybean oil meal – Poekstrakcyjna śruta sojowa	247	241	230
NaCl	2.1	2.1	2.1
Monocalcium phosphate – Fosforan jednowapniowy	12.5	12.0	12.0
Limestone – Kreda pastewna	54	55	55
DL-Methionine (technically pure) – DL-metionina (czysta technicznie)	1.0	1.9	1.9
L-Lysine hydrochloride – Chlorowodorek lizyny	2.0	2.0	2.0
Kemzyme DRY X [a]	0.5	0.5	0.5
Toxfin [b]	1.0	1.0	1.0
Premix DJR [c]	5.0	5.0	5.0
Premiks DJR [c] Sodium hydrogen carbonate – Wodorowęglan sodu	2.0	2.0	2.0
Natuphos 5% Layer [d]	2.0	2.0	2.0
Chemical composition – Skład chemiczny			
Dry matter – Sucha masa	883	883	884
ME (MJ) – EM (MJ)	11.7	11.7	11.7
Crude protein – Białko ogólne	194.0	195.0	195.0
Crude fiber – Włókno surowe	37.5	40.0	42.7
Calcium – Wapń	25.0	25.2	25.1
Available phosphorus – Fosfor przyswajalny	5.71	5.56	5.51
Lysine – Lizyna	10.9	10.6	10.3
Methionine – Metionina	4.03	4.13	4.16
Methionine + cysteine – Metionina + cystyna	7.55	7.51	7.40

[a] Dry stabilized enzyme preparation containing α-amylase, β-xylanase, β-glucanase.
[a] Suchy stabilizowany preparat enzymatyczny zawierający α-amylazę, β-ksylanazę, β-glukanazę.
[b] Preparation against mycotoxins.
[b] Preparat przeciw mykotoksynom.
[c] Vitamin-mineral premix.
[c] Premiks witaminowo-mineralny.
[d] Enzyme preparation containing phytase.
[d] Preparat enzymatyczny zawierający fitazę.

composition is probably due to a different BCS cultivation origin. Major components of fatty acids (FA) were linoleic (C18:2 n-6) and oleic (C18:1 n-9) acids. The dominant FA was linoleic acid, which accounted for 53.48% of the total FA.

Egg production and feed intake

Introduction of BCS into the diet in the amount of 20 and 50 g · kg^{-1} did not affect EP and EW (Table 3). Previously published studies show that the use of black cumin in feeding quail has a positive effect on their laying performance and egg mass [Denli et al. 2004, Szczerbińska et al. 2012]. Similar results were obtained in studies on chickens [Akhtar et al. 2003]: hens offered 15 g · kg^{-1} BCS attained much higher egg production compared to the control (production increased from 59 to 77%). Also, a higher egg weight was reported. In the study by Aydin et al. [2008], hens fed diets with addition of 30 g · kg^{-1} BCS were also characterized by better egg laying performance. Furthermore, a considerable increase in egg weight was observed in the groups obtaining a diet with addition of 10, 20 and 30 g · kg^{-1} BCS when compared to the control group. The enhanced performance of laying hens under BCS-supplemented feeding may be associated with phytosterols, as well as with the stimulators of insulin secretion (precursors and activators of hydroxyisoleucine), which promote protein synthesis and improve transmembrane transport into cells of glucose, amino acids, potassium and fatty acids [Asshayerizadem et al. 2009]. These mechanisms can have a positive impact on the vitellogenesis and egg white deposition.

The findings of the cited authors considerably differ from the results obtained by El Bagir et al. [2006], who observed that egg production decreased after application of 10 and 30 g · kg^{-1} BCS. The authors suggest that it may have been caused by a considerable decrease in yolk cholesterol level in the eggs of layers fed BCS.

Table 2. Composition of BCS

Tabela 2. Skład BCS

Ingredient – Składnik	g · kg^{-1}
Dry matter – Sucha masa	928.60
Crude protein – Białko ogólne	248.60
Ether extract – Ekstrakt eterowy	319.20
Crude ash – Popiół surowy	43.60
Crude fiber – Włókno surowe	19.30
Composition of BCS oil, % of total of fatty acids Skład oleju BCS, % ogółu estrów metylowych kwasów tłuszczowych	
C14:0	0.29
C16:0	12.65
C17:0	0.06
C18:0	3.21
C16:1	0.85
C18:1 n-9	23.37
C20:0	1.20
C20:1	0.33
C18:2 n-6	53.48
C20:2	4.27
C18:3 n-3	0.29
Total SFA – Ogółem SFA	17.41
Total MUFA – Ogółem MUFA	24.55
Total PUFA – Ogółem PUFA	58.04
Total UFA – Ogółem UFA	82.59

Along with an increasing percentage of the experimental component in the diet, we found a lower FI and improved FE. The lower feed consumption in the groups offered the diet with BCS in our study may be explained with its worse palatability due to the bitter taste. It is believed that the sense of taste in Japanese quails is poorly developed, but the study by Mills et al. [1997] proves that the birds prefer the sweet taste. The health status of birds during this experiment does not raise any objections, while deaths in the control group were of accidental nature (Table 3).

In the study by Denli et al. [2004], feed efficiency was higher in the group consuming 1 g · kg^{-1} BCS extracts. However, there were no differences for FI among birds fed treatment diets. In the study by Yalcin et al. [2009], FE in hens fed a feed supplemented with 10 and 15 g · kg^{-1} BCS was considerably improved. Similar results were obtained in the study by Cetin et al. [2008], who observed that FI decreased linearly along with a growing percentage of black cumin seed extract in the feed ration of partridges. Also, in the study by Abu-Dieyeh and Abu-Darwish [2008] on broiler chickens, an addition of 15 g · kg^{-1} BCS improved FE compared to the control group. More efficient feed utilisation might be associated with an antimicrobial effect of BCS in the gastrointestinal tract. Moreover, essential oils contained in BCS could have contributed to a better nutrient digestibility through stimulation of gizzard and pancreas. Our results indicate that the discussed ratio was more favourable in the groups receiving black cumin. This suggests, therefore, that phytosterols and stimulators of insulin secretion in BCS improved feed digestibility, ensuring its better utilisation. In has been demonstrated in previous studies that broiler chickens fed a black cumin powder-supplemented feed attained the highest final body weight, lower feed consumption and the best feed utilisation [Abu-Dieyeh and Abu-Darwish 2008, Ashayerizadeh et al. 2009].

Yolk fatty acid profile and cholesterol content

Our results indicate that egg yolks in the groups receiving 20 and 50 g · kg^{-1} of BCS in their diet were characterised by a significantly lower percentage of SFA (Table 4). In the case of monoenoic FA, their percentage in egg yolk lipids in groups receiving 20 and 50 g · kg^{-1} of BCS distinctly decreased. With an increasing percentage of the experimental component in the diet, a significant increase in the level of UFA was observed, by 1 and 3% respectively, including polyenoic acids by 7 and 14% respectively, as compared to the control.

The results of this study indicate that egg yolks from the laying hens receiving BCS in their diet were characterised by a higher percentage of linoleic acid (C18:2 n-6), linolenic acid (C18:3 n-3), arachidonic acid (C20:4 n-6) and eicosatrienoic acid (C20:3 n-3). On the other hand, no significant differences were observed in docosahexaenoic acid (DHA) (C22:6 n-3), which is different from the results obtained by Yalcin et al. [2009]. The percentage of DHA acid in that experiment was higher in groups offered 10 and 15 g · kg^{-1} BCS in their diet. PUFAs play an important role in the proper course of many vital functions in the human body [Milner and Allison 1999]. This is particularly true for arachidonic, DHA and eicosapentaenoic acids which, as components of nervous tissue phospholipids (cerebral cortex and retina), play a fundamental role during the prenatal life and early childhood, and are also precursors of eicosanoids, important tissue hormones. The percentage of PUFA from the n-3 family did not change, whereas that of n-6 fatty acids, as well as the ratio of total polyenoic belonging to the n-6 and the n-3 families, were significantly higher in test groups (19.57 and 22.56, respectively), as compared to the control, i.e. 14.8 (Table 4). Appropriate proportion of n-3 and n-6 fatty acids in diet is important. An excess of omega-6 fatty acids in the diet with a deficiency in omega-3 increases susceptibility to allergies and cancer, as well as to cardiovascular, nervous system and metabolic diseases. It is necessary to consume the right proportions of both types of fatty acids, if we are to keep good health. Group n-6 FAs (especially arachidonic acid) participate in the production of pro-inflammatory hormones. By contrast, n-3 fatty acids inhibit inflammation because they compete with n-6 fatty acids, decreasing their tissue concentration and reducing their reactions with enzymes [Gallia and Calderb 2009].

Table 3. Effect of BCS on the performance parameters of laying quails (from 8 to 23 weeks of age, x̄ ±SD)

Tabela 3. Wpływ BCS na wyniki użytkowości nieśnej przepiórek (od 8. do 23. tygodnia życia, x̄ ±SD)

Item	BCS supplementation, g · kg^{-1} – Dieta z udziałem BCS, g · kg^{-1}		
	0	20	50
Egg weight, g – Masa jaja, g	11.82 ±0.49	11.88 ±0.46	11.85 ±0.43
Egg production, % – Nieśność, %	84.1 ±7.80	86.0 ±6.70	85.1 ±7.54
Feed intake, g · day^{-1} per bird – Spożycie paszy, g · dzień$^{-1}$ na ptaka	36.21^{A}±4.08	33.31^{B}±2.89	30.93^{C}±3.71
Feed efficiency, kg feed per kg egg – Zużycie paszy, kg paszy na kg jaj	3.69^{A}±0.05	3.28^{B}±0.03	3.10^{C}±0.03
Deaths and culling, n – Upadki i brakowania zdrowotne, n	2	0	0

^{ABC} values within a row followed by different superscripts differ significantly (P < 0.01).
^{ABC} wartości w wierszu oznaczone różnymi literami różnią się istotnie (P < 0,01).

Table 4. Effect of BCS on total cholesterol content and yolk fatty acids profile (x̄ ±SD)

Tabela 4. Wpływ BCS na ogólną zawartość cholesterolu i profil kwasów tłuszczowych żółtka (x̄ ±SD)

Item	BCS supplementation, g · kg^{-1} – Dieta z udziałem BCS, g · kg^{-1}		
	0	20	50
Yolk cholesterol, mg · g^{-1} yolk – Cholesterol w żółtku, mg · g^{-1} żółtka	13.57^{A} ±0.15	13.28^{B} ±0.14	13.03^{C} ±0.16
Fatty acids, % – Kwasy tłuszczowe, %			
C14:0	0.24^{A} ±0.01	0.19^{B} ±0.01	0.19^{B} ±0.02
C16:0	29.70^{A} ±0.28	28.30^{B} ±0.14	26.06^{C}±0.18
C17:0	0.105^{A}±0.005	0.104^{A}±0.003	0.125^{B}±0.001
C18:0	3.55^{A}±0.08	4.04^{B}±0.07	4.06^{B}±0.03
C14:1	0.31^{A}±0.03	0.15^{B} ±0.01	0.10^{C}±0.001
C16:1	14.68^{A}±0.40	11.71^{B} ±0.23	8.54^{C}±0.08
C18:1 n-9	19.30^{A}±0.66	17.20^{B} ±0.19	16.38^{C}±0.25
C18:1 n-9t	5.48^{A}±0.06	4.81^{B} ±0.04	4.18^{C}±0.03
C20:1	0.094^{A}±0.006	0.101^{B}±0.003	0.094^{A} ±0.002
C18:2 n-6	20.24^{A}±0.28	25.97^{B}±0.20	32.61^{C}±0.36
C18:2 n-6t	1.02^{A}±0.06	1.36^{B}±0.12	1.19^{C}±0.16
C20:2n-6	0.028^{A}±0.004	0.068^{B}±0.006	0.114^{C}±0.004
C18:3 n-3	0.205^{A}±0.02	0.234^{B}±0.003	0.286^{C}±0.005
C20:3 n-3	0.14^{A}±0.01	0.18^{B}±0.01	0.17^{B}±0.01
C20:4 n-6	3.40^{A}±0.14	4.25^{B}±0.09	4.50^{C}±0.10
C20:5 n-3	0.204^{A}±0.01	0.176^{B}±0.003	0.160^{C}±0.004
C22:6 n-3	1.13^{A} ±0.09	1.03^{A} ±0.02	1.13^{A} ±0.03
Total SFA – Ogółem SFA	33.84^{A}±0.33	32.71^{B}±0.18	30.50^{C} ±0.19
Total MUFA – Ogółem MUFA	39.88^{A}±0.47	33.99^{B}±0.25	29.30^{C}±0.29
Total PUFA – Ogółem PUFA	26.45^{A}±0.48	33.32^{B} ±0.20	40.21^{C}±0.40
Total UFA – Ogółem UFA	66.33^{A}±0.34	67.31^{B}±0.18	69.52^{C} ±0.19
n-3	1.68^{A}±0.14	1.62^{A} ±0.03	1.70^{A} ±0.05
n-6	24.75^{A} ±0.38	31.63^{B} ±0.19	38.39^{C}±0.41
n-6/n-3	14.82^{A}±1.01	19.57^{B}±0.40	22.56^{C}±0.72

^{ABC} values within a row followed by different superscripts differ significantly (P < 0.01).
^{ABC} wartości w wierszu oznaczone różnymi literami różnią się istotnie (P < 0,01).

The analysis of total cholesterol indicates that the birds fed with 20 and 50 g · kg^{-1} of BCS accumulated its significantly lower amount in egg yolks as compared to the control. A decrease in the LDL fraction of cholesterol and the total content of that sterol in egg yolk has been also observed by Khan et al. [2013], who fed hens with BCS in the amount of 30, 40 or 50 g · kg^{-1}. Also, Akhtar et al. [2003] reported that an addition of 15 g · kg^{-1} BCS induces a decrease in total cholesterol level to 199.72 mg in egg yolk compared to the control (227.63 mg per egg yolk). On the other hand, Bölükbaşi et al. [2009], who studied hens fed a diet with 1, 2 and 3 ml of black cumin seed oil per 1 kg of feed observed a decrease in yolk total cholesterol concentration in the groups fed 1 and 2 ml of this oil per 1 kg of feed. At the same time, the authors found a higher percentage of cholesterol in the group receiving an addition of 3 ml black cumin seed oil per 1 kg of feed. BCS hypocholesterolemic properties are attributed to the reduction effect of HMG-CoA reductase, the rate controlling cholesterol synthesis in the liver due to the presence of essential oils in BCS [El-Dakhakhny et al. 2000].

CONCLUSIONS

In conclusion, the results of this study indicate that no statistically significant differences were observed in EW and EP, whereas FI decreased and FE improved in the groups receiving BCS in their diets. The health-promoting properties of a product being obtained are determined by the fact of a considerable decrease in cholesterol level in egg yolks. Introduction of BCS into the diet offered to quail affected the FA profile in egg yolks. A considerable increase was observed in the level of PUFA, especially in PUFA n-6, with a simultaneous decrease in SFA. Unfortunately, the n-6/n-3 ratio increased in groups fed BCS. Therefore, it can be concluded that under these circumstances eggs obtained from quails receiving BCS in the diet did not meet the requirements of functional food.

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WPŁYW DIETY ZAWIERAJĄCEJ CZARNUSZKĘ SIEWNĄ (*NIGELLA SATIVA*) NA PROFIL KWASÓW TŁUSZCZOWYCH I ZAWARTOŚĆ CHOLESTEROLU W ŻÓŁTKU JAJA PRZEPIÓRKI JAPOŃSKIEJ (*COTURNIX JAPONICA*)

STRESZCZENIE

Celem badań było ustalenie czy dieta z udziałem nasion czarnuszki siewnej (BCS) może być wykorzystana do produkcji jaj funkcjonalnych. Stado przepiórek liczące 96 samic, w wieku 8 tygodni podzielono na 3 grupy żywieniowe. Grupę kontrolną (0 BCS) żywiono paszą bez dodatku czarnuszki siewnej. Do diety ptaków z grup doświadczalnych dodano BCS, w ilości odpowiednio: 20 i 50 g $\cdot$ kg^{-1}. Wprowadzenie czarnuszki do diety przepiórek obniżyło spożycie paszy, poprawiając natomiast jej wykorzystanie. W żółtkach jaj przepiórek żywionych paszą z BCS stwierdzono mniejszy udział SFA i większy UFA. Istotnie wzrosła zawartość wielonienasyconych kwasów tłuszczowych (PUFA), szczególnie PUFA n-6. Na właściwości prozdrowotne jaj przepiórek żywionych dietą czarnuszkową, wskazywał fakt znacznego obniżenia zawartości cholesterolu w żółtku. Niestety stosunek kwasów n-6 do n-3 wzrósł znacząco w grupach żywionych dietą z udziałem BCS, i pod tym względem jaja nie spełniały wymagań żywności funkcjonalnej.

Słowa kluczowe: czarnuszka siewna, przepiórka japońska, żywienie, użytkowość, jaja funkcjonalne

Danuta Szczerbińska https://orcid.org/0000-0001-7970-3232
Danuta Majewska https://orcid.org/0000-0001-7013-1151
Michalina Adaszyńska-Skwirzyńska https://orcid.org/0000-0002-6729-845X