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ORIGINAL ARTICLE

Impact of bee bread supplementation on Japanese quails: laying performance, eggshell chemical composition and serum biochemistry

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Abstract
Bee bread is exceptional product of the beehive by its composition and a unique production by honeybees. Since the legislation prohibits the use of growth stimulants in animal husbandry, there is a growing interest in improving the yield of meat and eggs, and its quality parameters after applying various natural products. The impacts of bee bread supplementation on laying performance, eggshell chemical composition, serum biochemical parameters of Japanese quails were studied. Antioxidant activity, polyphenols, flavonoids, phenolic acids from bee bread, feeding mixture and combination was determined. A total of 45 female quails were involved in the experiment. The quails were divided into three groups as follows: group with 0.2% addition of bee bread into feeding mixture (E1, $n = 15$), group with 0.6% addition of bee bread into feeding mixture (E2, $n = 15$), and the control without additives (C, $n = 15$). The groups were kept under the standard conditions. After 180 days the animals were slaughtered, blood samples were collected. Addition of 0.6% bee bread in group E2 significantly decreased TAG level compared to group E1, without affecting laying performance. We noticed significant increase in Cd, Pb levels in eggshell in group E1 compared to control group. On the other side, 0.6% addition of bee bread caused significant reduction in Pb, Cd levels in eggshell compared to group with 0.2% addition of bee bread. The incorporation of bee bread into feeding mixture added improved antioxidant activity along with polyphenols and flavonoids. The results indicate that the effect of bee bread was dose dependent. The effective dosage estimation of additives used in feed for Japanese quails plays important role.

Keywords Antioxidants · Serum biochemistry · Japanese quails · Laying performance · Mineral content

Introduction

In recent decades, there has been an increased advocacy for the utilization of botanical and natural compounds as antibiotic alternatives, aiming to enhance both the performance and the immunological defenses of livestock. The European Union's ban on antibiotics in animal feed has led to an increase in research aiming for finding alternative substances that can safely improve animal health and growth (Casewell et al. 2003; Griggs and Jacob 2005; Khan et al. 2019; Bai and Li 2022). Incorporating a variety of natural compounds, ranging from medicinal herbs, vegetables, edible fungi, spices, seeds, invertebrates, apicultural products, other chemical compounds into the diets of Japanese quails, may confer beneficial effects on the qualitative attributes of the produced meat and eggs, primarily through the mitigation of oxidative stress mechanisms. However, this effect depends on the concentration and type of ingredients,

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Table 1 Declared nutritional composition of feeding mixture HYD 10

Parameter	Amount
Moisture	> 10%
Nitrogenous substances	72%
Fat	> 10%
Ashes	13%
NaCl	3%
(E324) Ethoxyquin	150 mg.kg^{-1}

Table 2 Chemical feed composition for feeding mixture HYD 10

Composition	Declared quality features
Corn 32%	nitrogenous substances min 200 g.kg^{-1}
Extracted soybean meal 19.2%	fibre max 60 g.kg^{-1}
Wheat 15%	ash max 160 g.kg^{-1}
CaCO3 10%	ME min 11.7 MJ.kg^{-1}
Rapeseed meal 7%	lysine min 7.5 g.kg^{-1}
Sunflower meal 4.5%	methionine and cysteine min 6 g.kg^{-1}
Animal fat 4%	linoleic acid min 10 g.kg^{-1}
Malt flower 3%	Ca min 35 g.kg^{-1}
Monocalcium phosphate 1%	P min 5 g.kg^{-1}
Premix additives 1%	Na min 1.6 g.kg^{-1}
NaCl 0.3%	
Fish meal 3%	

conformation of the compounds present in the source (Vargas-Sánchez et al. 2019). Possible natural alternative for this purpose might be bee bread. Bee bread is a product of apiculture, formed through anaerobic fermentation within the hive's cells. This substance comprises pollen foraged by bees, nectar or honey, and apian digestive secretions, which undergo biochemical transformation (Kieliszek et al. 2018). Bee bread is a nutrient-rich apicultural product encompassing all vital amino acids and a spectrum of vitamins, including those from the B-complex and vitamin K. It also serves as a substantial source of minerals such as potassium (K), magnesium (Mg), sodium (Na), calcium (Ca), phosphorus (P), and manganese (Mn). Bee bread is composed of a variety of fatty acids and carbohydrates, as well as a range of antioxidative molecules, including flavonoids (Čeksterytė et al. 2006; DeGrandi-Hoffman et al. 2013; Zuluaga et al. 2015; Kieliszek et al. 2018) as well as important omega-3 linolenic acid (ALA) (Čeksterytė et al. 2012). Based on Zuluaga et al. (2015) findings, bee bread exhibits a significant potential for bioavailability and utility. The experimental animal used in this study was the Japanese quail (*Coturnix japonica japonica* Temminck & Schlegel, 1848) (Padgett and Ivey 1959) first established as a research model by Padgett and Ivey in 1959. Quails possess several advantages for breeding and production. They have a more rapid generational turnover, reach maturity sooner, and have lower requirements for feed and space. Additionally, quails demonstrate a notable resistance to many of the diseases that commonly afflict chickens (Vali 2008; Santos et al. 2011; Sakamoto et al. 2018). The aim of the present study was to determine the effects of different dosses of bee bread supplementation on laying performance, mineral composition of eggshell, blood biochemical parameters of Japanese quails (*Coturnix japonica*) and antioxidant activity of bee bread, feeding mixture and combination.

Materials and methods

Livestock and diet

A total number of 45 female Japanese quails were used in the experiment. Birds were housed at the Research Institute of Animal Production in Nitra, using a cage system with a ratio of four animals per cage (0.12 m^2 area). In addition to water and feed consumption, quails were fed on *ad libitum* basis a standard basal diet (HYD 10, Tekro, Slovak Republic) providing 9 MJ.kg^{-1} ME (metabolised energy) balanced to meet the dietary needs (National Research Council, 1994). The nutritional composition of the diet is presented in Tables 1 and 2. Experimental animals were divided to three groups according to addition of bee bread into feeding mixture as follows: C ($n = 15$) diet without supplementation of bee bread, P1 ($n = 15$) diet with 2 g (0.2%) of bee bread per 1 kg of feeding mixture, P2 ($n = 15$) diet with 6 g (0.6%) of bee bread per 1 kg of feeding mixture. Over the experiment (180 days), the animals were housed in a thermoneutral hall at environmental temperatures (21 ± 2°C and 64 ± 2% humidity). Thermo aggregate was installed in a closed room, and sensors were used to replicate experimental conditions with defined temperature and humidity ranges. Electronic recorders were used to continuously monitor the simulated conditions (Hivus s.r.o., Zilina, Slovak Republic). The slaughter of the quails took place in accordance with Government Decree no. 289/20,003 Coll. rapid decapitation at the Slovak University of Agriculture in Nitra.

Laying performance, eggshell chemical composition

Japanese quails start egg laying at seven weeks of age. Eggs were collected and laying was recorded daily over four months of laying period. Eggs with defects (broken shell) were eliminated. Eggs were collected and registered from each cage in the peak of laying interval (September, October) and chemical composition of eggshell was evaluated. The chemical composition of the shell was determined from the ash by an atomic absorption spectrophotometer (iCE 3000 Series Atomic Absorption Spectrometers). The concentrations of iron (Fe), zinc (Zn), copper (Cu), cadmium

(Cd), lead (Pb), magnesium (Mg), calcium (Ca) were measured in 2 g of wet weight of eggshell. Eggshell is firstly oven-dried (60 °C, SUP-100 W dryer, WAMED), then mineralized with hot nitric acid (65%, Baker Analyzed, JT Baker, Phillipsburg, NJ, USA) in the open mineralization system (Velp Scientifica DK20). Mineralized solutions were diluted with ultrapure water (18.2 MV.cm at 25 °C, Direct-Q 3, Merck-Millipore, Germany) up to 10 mL and analysed with a flame atomic absorption spectrometer (AAnalyst 200, Perkin Elmer, Waltham, MA, USA). The results, after comparison with the limits of quantification and recalculations, were presented as mg of metal per 1 g of the dry sample (mg.g^{-1} d.w.). The whole procedure was checked against the certified reference material analysis. Analysis of elements (Ca, Zn, Fe, Mn, K, Na, Mg, Ca) was carried out using atomic absorption spectrophotometer iCE 300 (iCE 3000 Series Atomic Absorption Spectrophotometeres) at set wavelengths: Cu– 324.8 nm; Zn– 213.9 nm; Fe– 248.3 nm; Mn– 279.5 nm; Mg– 279.5; Ca– 422.7 nm. Phosphorus content was measured by spectrophotometry at wavelength of 430 nm using M 501 Single Beam Scanning UV/Visible Spectrophotometer Camspec.

Serum biochemical analysis

The blood samples from euthanized animals were used for biochemical analyses (mineral profile, energy profile, lipid profile). The blood serum was separated by centrifugation (30 min, 3000 RPM). Biochemical parameters of mineral profile as calcium (Ca), magnesium (Mg), and phosphorus (P) were analysed using RX Monza biochemical analyser (Randox, United Kingdom). Sodium (Na), potassium (K) and chlorides (Cl) were analysed by EasyLite microprocessor (Medica, Bedford, MA, USA). To analyse parameters of energy profile (glucose, total proteins), lipid parameters (total cholesterol, triglycerides) biochemical analyser RX Monza (Randox, United Kingdom) was used. The values of HDL and LDL cholesterol were measured using commercial kits by Genesys 10 (Thermo Fisher Scientific Inc., USA) at wavelength of 600 nm.

Determination of antioxidant activity by DPPH method

For the determination, 3.6 ml of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical (Sigma Aldrich, GE) and 0.4 ml of water was pipetted into the cuvette. The mixture was stirred rapidly and placed in the dark for 10 min, followed by a decrease in absorbance on a spectrophotometer (Jenway 6405 UV/Vis, UK) at 515 nm. The determination was performed in triplicate. The antioxidant activity was expressed based on the Trolox calibration curve (TEAC) in mg TEAC.g^{-1} dry matter ($R^2 = 0.9881$). Antioxidant activity was evaluated in the bee bread, feeding mixture and combination of feeding mixture with 0.4% bee bread.

Determination of total polyphenols by Folin - Ciocalteu method

For the analysis we pipetted 0.1 ml of the sample into the tubes and then add 0.1 ml of Folin - Ciocalteau reagent, 1 ml of 20% Na_2CO_3 solution and 8.8 ml of distilled water. The samples were mixed and after 30 min the absorbance (Jenway 6405 UV/Vis, UK) was measured at 700 nm against a blank. The total polyphenol content was expressed based on the gallic acid calibration curve in mg GAE.g^{-1} of sample ($R^2 = 0.9978$). Total polyphenols were evaluated in the bee bread, feeding mixture and combination of feeding mixture with 0.4% bee bread.

Determination of total flavonoids

0.5 ml of each sample and 0.1 ml of 10% ethanolic AlCl3 solution, 0.1 ml of 1 M sodium acetate and 4.3 ml of distilled water was pipetted into the tubes. The reaction mixture was mixed thoroughly and the absorbance (Jenway 6405 UV/Vis, UK) at 415 nm was measured against a blank. The flavonoid content was expressed based on the quercetin calibration curve in mg QE/g of sample ($R^2 = 0.9977$). Total flavonoid content was evaluated in the bee bread, feeding mixture and combination of feeding mixture with 0.4% bee bread.

Determination of total phenolic acid content

For the analysis of phenolic acids we pipetted 0.5 ml of extract, 0.5 ml of 0.5 mol.dm^{-3} HCl, 0.5 ml of Arn's reagent (10% $NaNO_2$ + 10% Na_2MoO_4), 0.5 ml of 1 mol.dm^{-3} NaOH and 0.5 ml H_2O. The absorbance of the reaction mixture was measured at 490 nm. The phenolic acid content was expressed as caffeic acid equivalent (CAE) in mg.g^{-1} ($R^2 = 0.9996$). Total phenolic acid content was evaluated in the bee bread, feeding mixture and combination of feeding mixture with 0.4% bee bread.

Statistical analysis

The results were statistically processed with GraphPad Prism 8 (GraphPad Software Inc., La Jolla, CA, USA) using One Way ANOVA using Tukey's test, which consists of group comparisons. The obtained values were used to calculate the basic statistical characteristics and differences between the experimental groups and the control group (standard deviation, mean). Differences were compared at the statistical level $p < 0.05$; $p < 0.01$; $p < 0.001$.

Results

Laying performance

We found no significant difference in laying performance presented in number of eggs per one female per 1 month (Table 3) between the control group and experimental groups ($p > 0.05$) after supplementation of bee bread. Although in October we noticed insignificant increase between the control group (22.67 eggs per month) and experimental groups E1 (24.75 eggs per month) and E2 (24.71 eggs per month).

Table 3 Laying performance of selected months

Calendar month	C	E1	E2	Significance
August	25.33	23.92	25.08	NS
September	23.08	23.50	24.50	NS
October	22.67	24.75	24.71	NS
November	21.25	23.79	20.87	NS

Values are given as number of eggs per one quail; NS–not significant; C–control group without supplement of bee bread, E1–experimental group with diet of bee bread 2 g per 1 kg of feeding mixture, E2–experimental group with diet of bee bread 6 g per 1 kg of feeding mixture

Eggshell chemical composition

We noticed in eggshell chemical composition (Table 4) significant increase ($p < 0.05$) of Cu content between the experimental group E1 (5.61 ± 0.89 mg.g.^{1} d.w.) compared to the experimental group E2 (7.72 ± 0.23 mg.g.^{1} d.w.). Significant increase ($p < 0.01$) was noticed in Cd and Pb concentration between the control group (2.75 ± 0.29 mg.g.^{1} d.w.; 46.41 ± 3.07 mg.g.^{1} d.w.) and experimental group E1 (1.92 ± 0.21 mg.g.^{1} d.w.; 33.46 ± 5.23 mg.g.^{1} d.w.). Compared to the experimental group E2, there was significant decrease ($p < 0.05$) of Cd, Pb concentration in group E1 (2.59 ± 0.10 mg.g.^{1} d.w.; 45.35 ± 1.07 mg.g.^{1} d.w.).

Table 4 Chemical composition of eggshell

Parameter (mg.g.^{1} d.w.)	C	E1	E2	Significance
Fe	13.93 ± 1.28	11.50 ± 1.93	13.87 ± 0.14	NS
Zn	2.41 ± 0.21	2.17 ± 0.42	2.57 ± 0.20	NS
Cu	7.13 ± 0.72	5.61 ± 0.89	7.72 ± 0.23	E1:E2 *
Cd	2.75 ± 0.29	1.92 ± 0.21	2.59 ± 0.10	C:E1 ** E1:E2 *
Pb	46.41 ± 3.07	33.46 ± 5.23	45.35 ± 1.07	C:E1 * E1:E2 *
Mg	1647 ± 133.7	1736 ± 101.1	1699 ± 255.7	NS
Ca	53629 ± 1552	53191 ± 4484	53920 ± 1507	NS

Values are given as mean ± SD (standard deviation); *$p < 0.05$; **$p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; NS–not significant; C–control group without supplement of bee bread, E1–experimental group with 2 g per 1 kg of feeding mixture, E2–experimental group with 6 g per 1 kg of feeding mixture; Fe–iron, Zn–zinc, Cu–copper, Cd–cadmium, Pb–lead, Mg–magnesium, Ca–calcium

Serum biochemical parameters

Results of the effect of bee bread on selected biochemical parameters are showed in Table 5. Analysis showed significant decrease ($p < 0.05$) in triglycerides between experimental group E1 (14.54 ± 5.86 mmol.L^{-1}) compared to E2 (11.22 ± 6.11 mmol.L^{-1}. Slight insignificant decrease ($p > 0.05$) was recorded in the aspartate aminotransferase between the control group (3.52 ± 0.54 µkat.L^{-1}) and experimental group E2 (6.25 ± 3.57 µkat.L^{-1}). There were no significant differences in other selected blood serum parameters between the control group and the experimental groups after application of bee bread into feeding mixture of female Japanese quails.

Determination of antioxidant activity, total polyphenols, total flavonoids, total phenolic acid content

The results of the antioxidant activity determination, total polyphenols, flavonoids, and total phenolic acid in Table 6 showed, that bee bread used for incorporation into feeding mixture was a good source of bioactive compounds, namely polyphenols (74.88 ± 1.10 mg.GAE.g^{-1}), flavonoids (91.76 ± 32.81 mg.QE.g^{-1}), fenolic acid (15.02 ± 25.01 mg.CAE.g^{-1}). Statistically significant ($p < 0.05$) increase of polyphenols was detected in bee bread (74.88 ± 1.10 mg.CAE.g^{-1}) compared to feeding mixture alone (20.75 ± 0.26 mg.CAE.g^{-1}).

Discussion

Our results showed that the application of various doses of bee bread used in this study did not affect the laying performance of Japanese quails in selected calendar months. In contrast to our results, increase in laying performance in Sinai hens was noticed by Awad et al. (2013) after supplementation up to 1.5% of bee bread. Zeweil et al. (2016), Desoky et al. (2018) noticed significant increase in laying performance in poultry after supplementation of propolis and pollen in the diet. After propolis supplementation in the diet of Japanese quails, Şentürk et al. (2021) did not notice significant differences in laying performance. There was no effect on egg production after inclusion of bee pollen into

Table 5 Serum biochemistry of Japanese quails supplemented with/without bee bread

Parameter	C	E1	E2	Significance
TC ($mmol.L^{-1}$)	4.64 ± 0.79	6.74 ± 2.45	6.07 ± 2.38	NS
TAG ($mmol.L^{-1}$)	11.19 ± 3.84	14.54 ± 5.86	11.22 ± 6.11	E1: E2 *
HDL ($mmol.L^{-1}$)	2.37 ± 0.36	2.81 ± 0.46	2.84 ± 0.99	NS
AST ($\mu kat.L^{-1}$)	3.52 ± 0.54	4.07 ± 1.41	6.25 ± 3.57	NS
ALT ($\mu kat.L^{-1}$)	0.04 ± 0.02	0.07 ± 0.03	0.06 ± 0.02	NS
GGT ($\mu kat.L^{-1}$)	0.03 ± 0.02	0.05 ± 0.02	0.03 ± 0.02	NS
ALP ($\mu kat.L^{-1}$)	6.23 ± 2.93	6.62 ± 2.51	7.13 ± 3.81	NS
GLU ($mmol.L^{-1}$)	16.85 ± 2.10	15.65 ± 1.67	16.73 ± 4.44	NS
ALB ($g.L^{-1}$)	15.09 ± 1.26	14.73 ± 1.92	16.32 ± 2.20	NS
TP ($g.L^{-1}$)	41.28 ± 3.56	42.06 ± 5.53	47.54 ± 6.28	NS
U ($mmol.L^{-1}$)	0.59 ± 0.13	0.53 ± 0.13	0.56 ± 0.23	NS
UA ($\mu mol.L^{-1}$)	190.7 ± 34.16	237.5 ± 73.09	271.3 ± 76.50	NS
Ca ($mmol.L^{-1}$)	8.21 ± 1.42	8.23 ± 0.76	7.42 ± 2.37	NS
P ($mmol.L^{-1}$)	2.11 ± 0.61	2.67 ± 1.14	2.87 ± 1.46	NS
Mg ($mmol.L^{-1}$)	1.83 ± 0.29	2.07 ± 0.30	1.97 ± 0.68	NS
Na ($mmol.L^{-1}$)	147.5 ± 1.88	147.8 ± 5.17	143.5 ± 7.98	NS
K ($mmol.L^{-1}$)	3.94 ± 1.17	3.57 ± 0.71	3.87 ± 0.66	NS
Cl- ($mmol.L^{-1}$)	117.7 ± 3.67	115.5 ± 3.47	113.8 ± 7.06	NS

Values are given as mean ± SD (standard deviation); *$p < 0.05$; NS–not significant; C–control group without supplement of bee bread, E1–experimental group with 2 g per 1 kg of feeding mixture, E2–experimental group with 6 g per 1 kg of feeding mixture; AST–aspartate aminotransferase; ALT–alanine transaminase, GGT–γ-glutamyl transferase, ALP–alkaline phosphatase; GLU–blood glucose, ALB–albumin, TP–total protein, U–urea, UA–uric acid, Ca–calcium, P–phosphorus, Mg–magnesium, Na–sodium, K–potassium, Cl–chloride

feeding mixture in laying hens after twelve weeks (Demir et al. 2020). Significant increase in Cd and Pb concentration in the experimental group E1 compared to the control group was noticed in our study. In contrary, there was significant decrease in Cd, Pb concentration in eggshell in group E1 (0.2% addition of bee bread) compared to E2 (0.6% addition of bee bread). Korénekova et al. (2007) reported correlation between Cd supplementation and increased content of Cd in eggshell of Japanese quails as a method of excretion this toxic element. Despite the fact that the bee bread is as a good source of Ca (Bakour et al. 2022), we did not notice any significant changes in level of Ca in eggshell after bee bread supplementation in treated groups possibly because the dosage was insufficient to effect concentration of Ca in eggshell. Similar results in eggshell of Japanese quails after turmeric powder supplementation noticed Saraswati et al. (2016). All mean values of selected biochemical parameters were within the reference values for Japanese quail (Scholtz et al. 2009). Biochemical parameters were not significantly influenced after bee bread supplementation, except triglycerides. We noticed a significant decrease in triglycerides between experimental groups E1 compared to E2 with the addition of 6 $g.kg^{-1}$ of bee bread into feeding mixture. The results of Pang et al. (2018) and Zhou et al. (2018) revealed that a higher dose of bee bread reduces triglycerides and total cholesterol levels in poultry, presumably due to hypotriglyceridaemic and hypocholesterolemic properties of bee bread. Kaplan et al. (2016) concluded, that due to rich unsaturated fatty acid (oleic and linoleic acid) composition we can expect positive effects on lipid profile after supplementation. Based on our results, we can conclude that 0.6% addition of bee bread significantly lowered TAG compared to 0.2% of bee bread addition. Due to the different conditions of the localities, it is therefore impossible to obtain identical two bee bread samples by composition. This is one of the reasons why the results of studies on the effects of bee bread on the body may differ. Kalafová et al. (2014) documented inconclusive effect on TAG and TC values after propolis and bee bread supplementation in broiler chicken after 42 days of feeding period. Awad et al. (2013) noticed after bee bread supplementation in concentration 0.5, 1 and 1.5 g per 1 kg of feeding mixture in Sinai hens insignificant decrease

Table 6 Antioxidant activity, total polyphenols, flavonoids, phenolic acid content

Parameter	BB	FM	BB + FM	Significance
Antioxidant activity ($mg. TEAC.g^{-1}$)	7.13 ± 1.05	6.73 ± 0.04	7.32 ± 0.41	NS
Polyphenols ($mg.GAE.g^{-1}$)	74.88 ± 1.10	20.75 ± 0.26	51.09 ± 27.30	BB:FM *
Flavonoids ($mg.QE.g^{-1}$)	91.76 ± 32.81	39.40 ± 10.00	72.83 ± 6.04	NS
Fenolic acid ($mg.CAE.g^{-1}$)	15.02 ± 25.01	5.62 ± 3.25	18.29 ± 1.82	NS

Values are given as mean ± SD (standard deviation); BB-bee bread; FM-feeding mixture; BB + FM-bee bread + feeding mixture in TAG. Farag - Rayes (2016) recorded a reduction of TC and TAG levels in the blood of broiler chickens using the addition of pollen. Effect on reduction of TC and TAG was noticed after supplementation of propolis in Japanese quails (Zeweil et al. 2016). Significant reduction in TAG levels after powdered turmeric (*Curcuma longa*) supplementation in Japanese quails was noticed by Putra et al. (2015). Bee bread might be responsible for the decrease in triglycerides and cholesterol because it acts as a powerful antioxidant, increasing the glutathione enzyme's activity, or because it contains components like essential fatty acids that inhibit the activity of the enzyme HMG-CO A reductase, a crucial regulator of cholesterol synthesis (Crowell 1999). Changes in activity of liver enzymes among the groups remains insignificant. We monitored the nitrogen profile (total protein, albumin, urea and uric acid) in quail females, where we did not notice any significant differences between the groups after bee bread supplementation. After evaluating the results of minerals in the blood serum, after 25 weeks we did not notice any significant changes in the monitored parameters (calcium, phosphorus, magnesium, sodium, potassium and chlorides) after the application of bee bread in the feed mixture in concentrations of 2 and 6 $g.kg^{-1}$, even in comparison with the control group. Our results correspond with results of Sevim (2021) in the study with bee pollen and growing quails. In contrary, Olgun et al. (2021) noticed significant, dose dependent, increase in serum Ca and P in poultry treated with bee pollen. Antioxidant properties relate to the presence of polyphenol compounds, and they have the ability to scavenge free radicals (Kapadiya et al. 2016; Kalwa and Wyrostek 2018). The mechanism of antioxidant activity is constantly being investigated and the effect on many mechanisms of modulation of the organism is analysed (Slobodnikova et al. 2016; Dziedziński et al. 2020). The content of the biologically active compounds is influenced by various factors such as technological processes, environmental conditions such as climate, therefore the number of bioactive compounds can vary (Dorozko et al. 2019). Compared to our results (7.33 $mg.TAC.g^{-1}$) Beykaya et al. (2021) recorded higher antioxidant activity (20.03–35.43 mg $TEAC.g^{-1}$) in Anatolian bee bread. By comparison with Ivanišová et al. (2019), the antioxidant activity ranged from 1.52 $mg.TEAC.g^{-1}$ in *Mespulus germanica* to 6.43 $mg.TEAC.g^{-1}$ in *Cornus mas*. The content of polyphenols in the bee bread used in our research was 74.88 $mg.GAE.g^{-1}$. Ivanišová et al. (2015) evaluated the content of total polyphenols in the bee bread of the Kiev region in 2015 in range of 12.36–18.24 mg $GAE.g^{-1}$. A wider range from 2.5 to 37.15 $mg.GAE.g^{-1}$ was documented by Sawicki et al. (2022) in the bee bread from Poland. As we can clearly notice, there is wide range of polyphenols obtained in bee breads from different locations. The content of polyphenols also varies considerably from the length and storage conditions. Mayda et al. (2020) analysed values of flavonoids in bee pollen from different locations and the values ranged from 1.81 ± 0.040 to 4.44 ± 0.125 mg $QE.g^{-1}$. The value of flavonoids in bee bread used in our experiment ranged in interval 91.76 ± 32.81 $QE.g^{-1}$. The addition of bee bread into feeding mixture might positively influence the nutritional value of the basal diet for Japanese quails.

Conclusions

Based on the results of present study, addition of bee bread in dosage of 0.6 $g.kg^{-1}$ was effective in lowering TAG blood level compared to the experimental group with 0.2 $g.kg^{-1}$ addition of bee bread without affecting laying performance of Japanese quails (*Coturnix japonica*). We noticed significant increase in Cd, Pb in experimental group with 0.2 $g.kg^{-1}$ addition of bee bread compared to the control group. On the other side, 0.6 $g.kg^{-1}$ addition of bee bread caused significant reduction in Pb, Cd compared to group with 0.2 $g.kg^{-1}$ addition of bee bread. Bee bread is rich source of polyphenols and flavonoids, and therefore has a strong antioxidant activity. The incorporation of bee bread into feeding mixture increased antioxidant activity along with polyphenols and flavonoids. For further investigation, we recommend analysing toxic elements from bee bread and feeding mixture, along with using wider dosage range of bee bread incorporated into feeding mixture. The obtained results of our work can contribute to the deepening and expansion of knowledge of bee products.

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Declarations

Conflict of interest All authors declare that they have no conflict of interest.

Ethics approval In this article all applicable international, national and institutional guidelines for the care and use of animals were followed.

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