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Research article

THE EFFECTS OF PROPOLIS AND BEE POLLEN SUPPLEMENTATION ON BIOCHEMICAL BLOOD PARAMETERS OF BROILERS

KLARIC Ivana[1], MISKULIN Ivan[2], SERIC Vatroslav[3], DUMIC Albina[2], JONJIC Jasna[3], MISKULIN Maja[2*]

[1]Department for Animal Husbandry, Faculty of Agriculture in Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia; [2]Department of Public Health, Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia; [3]Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

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The aim of this study was to determine the effect of propolis and bee pollen on selected biochemical blood parameters in broiler chickens. This experimental study was conducted on 200 Ross 308 chickens of equally distributed sex, which were divided into five groups (a control group and four experimental groups). Throughout the whole study the control group of chickens was fed the control feed mixture while the feed mixture that was fed to the experimental groups of chickens contained additives (propolis and/or bee pollen, each supplemented separately or in combination in a certain proportion). The results obtained by this study are: significantly lower blood glucose, cholesterol and calcium levels ($P < 0.001$), as well as significantly lower triglycerides levels ($P = 0.002$), but also significantly higher sodium and chloride ($P < 0.001$), phosphorus ($P = 0.004$) and globulins levels ($P = 0.027$) in chickens of the experimental groups compared to the chickens of the control group on the 21st day of fattening. Furthermore, this study has found significantly lower blood glucose ($P = 0.033$) levels and significantly higher levels of total proteins and globulins ($P = 0.003$), as well as albumins ($P = 0.040$) in chickens of the experimental groups compared to the chickens of the control group on the 42nd day of fattening. It can be concluded that the application of propolis and bee pollen as additives in broiler feeds enables the production of more vital and healthier animals, which significantly improves the fattening of chickens.

Key words: additives, blood, broilers feeding, feed supplements, health

INTRODUCTION

Propolis and bee pollen belong to a group of natural substances of animal and plant origin with particularly expressed antioxidant and antimicrobial properties [1]. The bioactive components of propolis and bee pollen include flavonoids, phenolic acids

*Corresponding author: e-mail: maja.miskulin@mefos.hr

and their derivatives which are also responsible for the bactericidal, antiviral, antifungal, analgesic, anti-inflammatory, antioxidant, immunostimulating and immunomodulating effects of these compounds in humans and animals [1-4]. In addition to these effects, it is demonstrated how propolis and bee pollen decrease the risk of atherosclerosis [5]. The high antioxidant activity of these compounds is connected with the content of flavonoids, protects internal organs (liver, kidney) from damage (mainly caused by toxic agents), stimulating the regeneration of damaged tissue, as well as showing potent anti-carcinogenic effects [3, 6].

Propolis has a long history of use which dates back to ancient times, some 300 years before Christ, and it has been used continuously until today [7, 8]. Propolis is used in many parts of the world as a favorite preparation in traditional medicine, in apitherapy, as an integral part of the so-called biocosmetics, healthy i.e. natural foods, and for a number of other purposes [9].

Bee pollen has been traditionally used as food which prevents aging and boosts energy [10]. Additionally, bee pollen can also enhance the cellular immune response, speed of antibody production and fully strengthen the immune system [11]. A large number of authors suggested in their research that propolis and bee pollen have a significant impact on production values and animal health as well [12-14].

The aim of this study was to determine the effect of propolis and bee pollen (each supplemented separately or in combination in a certain proportion), as additives in broilers feeding, on the values of selected biochemical blood parameters in broiler chickens.

MATERIAL AND METHODS

All treatments, housing and animal care were carried out in accordance with the standards recommended by EU Directive 2010/63/EU for animal experiments.

Animals and diets

This experimental study included a total of 200 day-old chickens of the Ross 308 provenance. The fattening trial of the chickens was carried out on a farm in Eastern Croatia under the supervision of the Department for Animal Husbandry, Faculty of Agriculture in Osijek, Josip Juraj Strossmayer University of Osijek. Total of 200 chickens of the Ross 308 provenance, evenly distributed sexes, were divided into 5 groups (40 chickens in each group), one of which was the control group (K) and the other four experimental groups (P1, P2, P3, P4). For the purpose of more effective monitoring of all the investigated indicators, on the seventh day of the trial all the chickens were marked with a leg ring.

During the study all the groups of chickens were fattened under the same conditions. Temperature, humidity and lighting in the facility were maintained within optimum limits according to the manufacturer's recommendations for Ross 308 hybrid [15].

Fattening was conducted on wooden sawdust, and lasted for 6 weeks (42 days). During the study, feed and water were given to chickens *ad libitum*.

Table 1. The composition and calculated analysis of feed mixtures used for chickens fattening

Fodder, %	Starter	Finisher
	1–21 day	22–42 day
Corn grain	45.00	46.10
White bran-mild	2.80	3.00
Dehydrated alfalfa	2.80	4.00
Soybean meal	20.20	10.00
Sunflower meal	4.00	4.00
Yeast	4.00	3.00
Full fat soybean	12.40	20.00
Vegetable oil	3.70	5.00
Monocalcium phosphate	1.20	1.20
Limestone	1.60	1.40
Salt	0.30	0.30
Premix*	1.00	1.00
Pigozen 801	1.00	1.00
Total	100.00	100.00
Calculated Analysis		
Crude protein, %	21.02	19.15
Crude fat, %	8.36	10.96
Crude fiber, %	4.96	5.05
Lysine, %	1.11	0.96
Methionine, %	0.66	0.61
Tryptophan, %	0.26	0.23
Calcium, %	1.04	0.98
Phosphorous, %	0.70	0.67
ME, MJ/kg	12.30	13.10

*Each 1 kg of premix contained: Vitamin A 1200000 IU; Vitamin D3 200000 IU; Vitamin E 3000 mg; Vitamin K3 250 mg; Vitamin B1 150 mg; Vitamin B2 600 mg; Vitamin B6 200 mg; Vitamin B12 1 mg; Folic acid 50 mg; Niacin 4400 mg; Ca Panthothenate 1500 mg; Biotin 10mg; Choline chloride 50000 mg; Iron 5000 mg; Copper 700 mg; Manganese 8000 mg; Zinc 5000 mg; Iodine 75 mg; Cobalt 20 mg; Magnesium 750 mg; Selenium 15 mg; Antioxidant BHT 10000 mg; Methionine 100000 mg; Herbal carrier 1000 g.

From days 1-21 of the study chickens were fed a starter mixture, and from days 22-42 of the study chickens were fed a finisher diet. The composition and calculated analysis

of feed mixtures used for fattening are shown in Table 1. Throughout the study the control group (K) of chickens was fed a standard feed mixture without additives, while experimental groups of chickens (P1, P2, P3, P4) were fed feed mixtures that contained additives – propolis and/or bee pollen as follows: P1 group: feed mixture + 0.25 g of propolis/kg of feed mixture + 20 g of bee pollen/kg of feed mixture; P2 group: feed mixture + 0.5 g of propolis/kg of feed mixture; P3 group: feed mixture + 1.0 g of propolis/kg of feed mixture; P4 group: feed mixture + 20 g of bee pollen/kg of feed mixture. Samples of raw propolis and multifloral bee pollen used in this study were obtained from apiaries located in naturally preserved areas of continental Croatia (around the city of Osijek). Propolis and bee pollen were crushed, and in powder form were mixed with dry feed mixture with the aid of a vertical mixer.

Sample collection and analysis

Blood sampling was performed twice during the study period (on days 21 and 42 of the study), on randomly selected chickens (10 birds from each group). Chickens that were selected for blood sampling on day 21 were used as experimental animals for monitoring of all the investigated parameters until the end of the study.

Blood sampling was performed by wing vein puncture (lat. *v. cutanea ulnaris*) with direct needle injection coupled with a test tube under vacuum. The collected blood samples were analyzed for the following biochemical parameters (Fe, Ca, Na, P, Mg, K, Cl, cholesterol, triglyceride, glucose, total proteins, globulins and albumins). In chickens' blood sera the values of the following parameters were determined: iron (Fe, μmol/L); calcium (Ca, mmol/L); sodium (Na, mmol/L); phosphorus (P, mmol/L); magnesium (Mg, mmol/L); potassium (K, mmol/L); chloride (Cl, mmol/L); cholesterol (CHOL, mmol/L); triglycerides (TRG, mmol/L); glucose (GLUC, mmol/L); total proteins (TP, g/L); globulins (GLO, g/L) and albumins (ALB, g/L). The values of the above mentioned biochemical parameters were determined using Olympus AU 680 automatic analyzer (Olympus Life Science Research Europa GmbH, Germany). Laboratory analyses of all the above mentioned blood parameters were performed at the Department of Clinical Laboratory Diagnostics, University Hospital Centre Osijek. For the purpose of interpretation of the obtained results of the analyses of the biochemical blood parameters the following reference values were used: Kaneko et al., Abdi - Hachesoo et al., Piotrowska et al. and Owosibo et al. [16-19].

Statistical analysis

Upon confirming normality of data distribution by Shapiro-Wilkinson test, all data were processed by the methods of descriptive statistics. The numerical variables were described as mean and standard deviation. The ANOVA and Kruskal-Wallis test were used for the comparison of numerical variables among the groups. The level of statistical significance was set at $P < 0.05$. Statistical analysis was done using statistical package Statistica for Windows 2010 (version 10.0, StatSoft Inc., Tulsa, OK).

Different lowercase letters at the level of statistical significance of $P < 0.05$ assigned to the individual values in the tables indicate a statistically significant difference while the same lowercase letters assigned to certain values in the tables indicate the absence or lack of statistically significant differences.

RESULTS

The values of the investigated biochemical parameters in chickens' blood on the 21st day of the fattening period according to the particular group of chickens are shown in Table 2. Statistical analysis has shown that there was a statistically significant difference in blood values of glucose, cholesterol, triglycerides, globulins, calcium, sodium, phosphorus and chloride while there was no statistically significant difference in values of total proteins, albumins, iron, magnesium and potassium between the groups of chickens on the 21st day of the fattening period.

Table 2. Biochemical parameters in chickens' blood on the 21st day of the fattening period

Parameters	Group of chickens $\bar{x} \pm sd$					P
	K	P1	P2	P3	P4	
GLUC (mmol/L)	$14.99^{a}\pm0.55$	$14.14^{ab}\pm0.87$	$13.37^{b}\pm0.65$	$13.94^{bc}\pm0.69$	$15.19^{ac}\pm1.07$	< 0.001*
CHOL (mmol/L)	$3.10^{a}\pm0.25$	$3.08^{a}\pm0.16$	$2.96^{a}\pm0.27$	$3.62^{b}\pm0.28$	$3.01^{a}\pm0.30$	< 0.001*
TRG (mmol/L)	$0.81^{a}\pm0.22$	$0.56^{b}\pm0.07$	$0.54^{b}\pm0.25$	$0.72^{a}\pm0.21$	$0.73^{a}\pm0.16$	0.002**
TP (g/L)	24.07±2.45	24.70±2.23	25.64±2.59	27.05±2.34	25.68±2.44	0.084*
GLO (g/L)	$14.44^{a}\pm1.43$	$15.25^{ac}\pm1.36$	$15.97^{bc}\pm1.95$	$16.76^{b}\pm1.42$	$15.78^{ab}\pm1.58$	0.027*
ALB (g/L)	9.63±1.11	9.45±0.96	9.67±0.86	10.29±0.99	9.90±0.91	0.364*
Fe (μmol/L)	13.37±1.58	11.57±1.61	11.82±4.10	11.34±4.14	14.97±3.21	0.064*
Ca (mmol/L)	$3.43^{a}\pm0.31$	$2.88^{b}\pm0.22$	$3.04^{b}\pm0.33$	$2.78^{c}\pm0.18$	$2.95^{bc}\pm0.17$	< 0.001*
Na (mmol/L)	$148.60^{ac}\pm1.71$	$150.20^{b}\pm2.86$	$150.90^{b}\pm1.20$	$148.40^{c}\pm0.97$	$147.60^{c}\pm1.17$	< 0.001*
P (mmol/L)	$1.17^{a}\pm0.29$	$1.44^{b}\pm0.19$	$1.41^{ab}\pm0.32$	$1.64^{b}\pm0.35$	$1.61^{b}\pm0.21$	0.004*
Mg (mmol/L)	0.92±0.75	0.86±0.05	0.89±0.08	0.83±0.06	0.87±0.06	0.114*
K (mmol/L)	4.75±0.24	4.70±0.43	4.56±0.44	4.44±0.35	4.49±0.31	0.257*
Cl (mmol/L)	$108.10^{a}\pm1.60$	$112.60^{b}\pm3.03$	$112.40^{b}\pm1.78$	$110.30^{ab}\pm2.11$	$108.00^{a}\pm1.49$	< 0.001*

*ANOVA; **Kruskal-Wallis test

$\bar{x}$ = mean; sd = standard deviation; means within rows without common superscripts differ significantly a,b,c $P < 0.05$; K = control group; P1 = feed mixture + 0.25 g of propolis/kg of feed mixture + 20 g of bee pollen/kg of feed mixture; P2 = feed mixture + 0.5 g of propolis/kg of feed mixture; P3 = feed mixture + 1.0 g of propolis/kg of feed mixture; P4 = feed mixture + 20 g of bee pollen/kg of feed mixture

The values of the investigated biochemical parameters in chickens' blood on the 42nd day of the fattening period according to the particular group of chickens are shown in

Table 3. Statistical analysis has shown that there was a statistically significant difference in blood values of glucose, cholesterol, total proteins, globulins, albumins, sodium and potassium while there was no statistically significant difference in values of triglycerides, calcium, phosphorus, magnesium, iron and chloride between the groups of chickens on the 42nd day of the fattening period.

Table 3. Biochemical parameters in chickens' blood on the 42nd day of the fattening period

Parameters	Group of chickens $\bar{x} \pm sd$					P
	K	P1	P2	P3	P4	
GLUC (mmol/L)	14.17^{ab}±0.81	14.66^{a}±0.98	13.80^{b}±0.51	13.62^{b}±0.56	13.93^{b}±0.52	0.033**
CHOL (mmol/L)	2.73^{a}±0.16	2.93^{ac}±0.28	2.84^{a}±0.23	3.36^{b}±0.28	3.11^{c}±0.35	<0.001*
TRG (mmol/L)	0.93±0.14	1.20±0.31	0.95±0.17	0.98±0.22	1.07±0.20	0.136**
TP (g/L)	28.31^{a}±3.10	27.18^{ac}±1.50	28.53^{ac}±1.93	30.82^{b}±2.65	30.86^{b}±2.42	0.003*
GLO (g/L)	17.69^{a}±1.98	16.77^{a}±0.76	17.85^{ac}±1.48	19.22^{bc}±1.65	19.34^{b}±1.71	0.003*
ALB (g/L)	10.62^{ac}±1.26	10.41^{a}±0.81	10.68^{ab}±0.96	11.60^{b}±1.23	11.52^{bc}±0.93	0.040*
Fe (μmol/L)	16.68±3.09	14.60±2.17	17.32±1.95	16.70±2.20	16.98±2.84	0.080**
Ca (mmol/L)	2.60±0.22	2.62±0.14	2.64±0.13	2.64±0.14	2.52±0.08	0.365*
Na (mmol/L)	150.10^{a}±2.08	149.90^{a}±1.29	152.00^{b}±1.89	152.00^{b}±1.76	149.40^{ab}±4.72	0.015**
P (mmol/L)	2.19±0.13	2.20±0.11	2.18±0.20	2.18±0.15	2.06±0.11	0.168*
Mg (mmol/L)	0.87±0.04	0.87±0.02	0.90±0.05	0.88±0.08	0.86±0.03	0.442*
K (mmol/L)	4.86^{a}±0.19	5.02^{a}±0.42	4.88^{a}±0.56	4.69^{a}±0.53	4.16^{b}±0.27	<0.001*
Cl (mmol/L)	112.20±1.87	112.20±1.14	113.60±2.07	113.50±1.58	112.70±5.14	0.235**

*ANOVA; **Kruskal-Wallis test
$\bar{x}$ = mean; sd = standard deviation; means within rows without common superscripts differ significantly a,b,c $P < 0.05$; K = control group; P1= feed mixture + 0.25 g of propolis/kg of feed mixture + 20 g of bee pollen/kg of feed mixture; P2 = feed mixture + 0.5 g of propolis/kg of feed mixture; P3 = feed mixture + 1.0 g of propolis/kg of feed mixture; P4 = feed mixture + 20 g of bee pollen/kg of feed mixture.

DISCUSSION

The present study has demonstrated that the dietary supplementation of broilers with propolis and/or bee pollen significantly alters the biochemical profile of their blood on the 21st and also on the 42nd day of fattening. These results are in line with the results of the study done by Omar et al. [20]. Regarding the values of total proteins and globulins in our study, as well as in the study done by Omar et al. [20], it was found that the values of total proteins and globulins in all the experimental groups (P1-P4) of chickens were higher than those determined in the control group of chickens.

The results of our study are opposite to those of the study of Shahryar et al. [21], who found that on the 42nd day of fattening there were no statistically significant differences

in the values of albumins and globulins in the blood between the experimental groups of chickens fed with the addition of various amounts of propolis and the chickens in the control group.

In their study, Tekeli et al. [22] showed that the chickens fed the diet with added propolis had on the 42nd day of fattening a significantly higher cholesterol level and a significantly lower triglyceride level in the blood as compared to the control group of chickens, while there were no statistically significant differences between the experimental and control groups of chickens in the values of blood glucose. Comparing these results with the results of our study it can be said that our results are only partially consistent.

Petruska et al. [23] reported that chickens fed with the addition of propolis had on the 42nd day of fattening a significantly lower level of phosphorus and magnesium in the blood in comparison with the chickens of the control group, while feeding with the addition of propolis did not affect the value of sodium, potassium, chloride and calcium in the blood of chickens. The results of our study are also partially consistent with the results of this study.

In their study, Attia et al. [24] have found that chickens in the experimental group fed with the addition of propolis and/or bee pollen had on the 35th day of fattening significantly lower cholesterol and triglyceride levels compared to the control group of chickens. These results are opposite to the results obtained in our study.

When analyzing the investigated biochemical parameters in chickens' blood in relation to the reference values of those parameters according to Kaneko et al., Abdi - Hachesoo et al., Piotrowska et al. and Owosibo et al. [16-19], it can be said that the values of the biochemical parameters observed in our study are generally consistent with the previously mentioned reference values.

This study further indicated that propolis and/or bee pollen significantly affected biochemical parameters in the blood of broiler chickens on the 21st and 42nd day of fattening. This study showed that the consumption of a feed mixture with the addition of propolis led to lowering blood glucose, cholesterol and triglyceride levels in the experimental group of chickens on the 21st day of fattening compared to the control group of chickens. The established ability of propolis to reduce the value of these biochemical parameters can be attributed to the regulatory mechanism of flavonoids, as one of the most important ingredients of propolis, for which it has been proven to affect blood circulation and the circulation of metabolites in the blood and to stimulate the use of triglycerides from the blood for energy generation. With regard to the lowering of blood glucose, it is considered that this effect resulted from the stimulatory effect of flavonoids on the release of insulin or insulin-like substances. Insulin stimulates glucose transport into liver cells which results in reduced blood glucose levels [22,25]. The explanation for lowering cholesterol levels is also associated with the effect of flavonoids that have been shown to lower blood cholesterol levels [25,26]. Considering the effect of propolis and bee pollen jointly, it can be said that

these compounds enhance lipid metabolism and have a positive effect on the liver and kidney function which is evidenced by lowering triglycerides and cholesterol levels in the blood [24]. Experts believe that the effect of bee pollen on plasma metabolites can be attributed to its vitamin and mineral composition, content of phospholipids in the bee pollen [27] and its antioxidant effects [6]. Also, it is believed that the effect of bee pollen in terms of lowering plasma cholesterol levels may be the result of the content of phospholipids and polyunsaturated fatty acids in bee pollen [28].

This study has revealed that at the end of the fattening period chickens in the experimental groups, especially chickens in P3 and P4 group, that consumed the feed mixture with the highest amount of added propolis and bee pollen, had significantly higher levels of total proteins, globulins and albumins in the blood, which may be due to the direct effect of these bee products on the anabolic processes in the context of protein synthesis, as a result of which body proteins are being protected against degradation [20].

Regarding the impact of propolis and bee pollen on the values of minerals in the blood of chickens, this study has proven that these additives affect the levels of the observed minerals, probably due to the fact that both of these additives are extremely rich in various minerals [11,29,30]. In addition, studies have shown that the addition of propolis in feed mixtures causes the increase in the levels of magnesium and phosphorus in the bones of chickens. The explanation of the previously mentioned effect of propolis lies in the fact that its addition to feed enhances absorption of phosphorus and magnesium from the blood into the bone, which consequently lowers the levels of these minerals in the blood [23, 31].

CONCLUSION

Propolis and bee pollen (either separately or in combination) have a significant positive impact on blood biochemical parameters in broilers. The application of propolis and bee pollen as additives in broiler feeding enables the production of more vital and healthier animals, which significantly improves the fattening of chickens. Considering all this, it can be said that the use of these additives is an innovative technological solution in broilers feeding.

Authors' contributions

IK, IM and MM made substantial contributions to conception and design of the manuscript, they participated in experimental design of the study and in the acquisition of the data and in the analysis and interpretation of data; they also drafted the article and gave final approval of the version of the manuscript to be published. VS and JJ participated in experimental design of the study and in the analysis and interpretation of data, they also revised article critically for important intellectual content and they gave final approval of the version of the manuscript to be published. AD made

substantial contributions to conception and design of the manuscript, she participated in the analysis and interpretation of data, she revised article critically for important intellectual content and she gave final approval of the version of the manuscript to be published.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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EFEKTI DODATKA PROPOLISA I POLENA NA BIOHEMIJSKE PARAMETRE U KRVI TOVNIH PILIĆA

KLARIC Ivana, MISKULIN Ivan, SERIC Vatroslav, DUMIC Albina, JONJIC Jasna, MISKULIN Maja

Cilj ovog istraživanja bio je da se ustanovi uticaj propolisa i polena na odabrane biohemijske parametre u krvi tovnih pilića. Ova eksperimentalna studija sprovedena je na 200 pilića Ross 308, ravnomerno raspoređenih polova, koji su bili podeljeni u pet grupa (kontrolna grupa i četiri eksperimentalne grupe). Tokom trajanja istraživanja kontrolna grupa pilića je hranjena čistom krmnom smesom za tov pilića, dok su krmnim smesama kojima su hranjene eksperimentalne grupe pilića dodani aditivi (propolis i/ili pčelinji polen, svaki od njih zasebno ili oba u kombinaciji u određenom odnosu). Rezultati istraživanja su: značajno niži nivo glukoze u krvi, holesterola i kalcijuma ($P < 0,001$), kao i značajno niži nivo triglicerida ($P = 0,002$), ali takođe znatno viši nivo natrijuma i hlorida ($P < 0,001$), fosfora ($P = 0,004$), te nivo globulina ($P = 0,027$) u krvi pilića eksperimentalnih grupa u poređenju s pilićima kontrolne grupe 21. dana tova. Dalje, ovo istraživanje je utvrdilo znatno niži nivo glukoze u krvi ($P = 0,033$) i znatno viši nivo ukupnih proteina i globulina ($P = 0,003$), kao i albumina ($P = 0,040$) u krvi pilića eksperimentalnih grupa u odnosu na piliće kontrolne grupe 42. dana tova. Može se zaključiti da primena propolisa i pčelinjeg polena kao aditiva u hrani tovnih pilića omogućuje proizvodnju vitalnijih i zdravijih životinja, što značajno poboljšava tov pilića.