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THE EFFECTS OF PROPOLIS SUPPLEMENTATION ON WEIGHT GAIN, FEED CONVERSION AND SELECTED BIOCHEMICAL BLOOD PARAMETERS OF BROILERS

Matija Domaćinović[1*], Ivana Prakatur[1], Ivan Miškulin[2], Daniela Čačić Kenjerić[3], Vatroslav Šerić[2], Berislav Prakatur[4], Maja Miškulin[2], Frane Čačić Kenjerić[3]

[1]Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia
[2]Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Josipa Huttlera 4, 31000 Osijek, Croatia
[3]Faculty of Food Technology Osijek, Josip Juraj Strossmayer University of Osijek, Franje Kuhača 20, 31 000 Osijek, Croatia
[4]Agricultural Food Company Valpovo Ltd., A. B. Šimića 27, 31550 Valpovo, Croatia

[*]e-mail: Matija.Domacinovic@fazos.hr

Abstract

High growth rate and feed efficiency are the two main targets in poultry production. For several decades, antibiotics have been widely used in the chicken diet. However, the use of antibiotics in broilers feeding has resulted in numerous problems and therefore their usage as a growth promoter is no longer acceptable and is prohibited in the European Union countries. Propolis as a natural substance pose itself as a valuable alternative in that sense. The aim of this study was to determine the effect of propolis supplementation on weight gain, feed conversion and selected biochemical blood parameters of broilers.

This experimental study was conducted on 180 Ross 308 chickens equally distributed by sex and divided into three groups: the control group of chickens (C) fed with a basal diet and two experimental groups of chickens (E) fed with the same diet supplemented with propolis (E1 2g/kg and E2 4g/kg). Blood sampling was performed by wing vein puncture with direct needle injection coupled with a test tube under vacuum on the 42th day of the study. The collected blood samples were analyzed for the following biochemical parameters (Fe, Ca, Na, P, Mg, K, Cl, cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, glucose, total proteins, globulins and albumins). The mentioned biochemical parameters were determined using Olympus AU 680 automatic analyzer (Olympus Life Science Research Europa GmbH, Germany). Laboratory analyses were performed at the Department of Clinical Laboratory Diagnostics, University Hospital Centre Osijek. 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 $p<0.05$.

The study revealed that there were no statistically significant differences in weight gain between C and E groups of chickens on 1st ($p = 0.547$), 2nd ($p = 0.464$), 3rd ($p = 0.792$), 4th ($p = 0.426$), 5th ($p = 0.925$) and 6th ($p = 0.971$) week of feeding. The study also showed that there were no statistically significant differences in feed conversion between C and E groups in any of the week of feeding ($p = 0.368$). Finally, the study revealed that there were statistically significant differences in Na levels ($p = 0.039$) and Ca levels ($p = 0.045$) while there were no statistically significant differences in HDL cholesterol ($p = 0.719$), LDL cholesterol ($p = 0.382$), cholesterol ($p = 0.462$), triglycerides ($p = 0.481$), proteins ($p = 0.249$), albumins ($p = 0.661$), globulins ($p = 0.058$), glucose ($p = 0.179$), Fe ($p = 0.068$), Mg ($p = 0.557$), K ($p = 0.590$), P ($p = 0.397$) and Cl ($p = 0.128$) levels between C and E groups.

It can be concluded that applied doses of propolis did not affect the observed performance indicators and selected biochemical blood parameters of broilers thus it is important to evaluate the effects of such supplementation through some other indicators.

***Key words**: Propolis, Broilers feeding, Weight gain, Feed conversion, Blood.*

1. Introduction

High growth rate and feed efficiency are the two main goals in modern poultry production. Sub-therapeutic doses of antibiotics were often used in livestock breeding to achieve these goals as well as to improve animal health and also to control pathogens effectively. However, due to the negative effects of antibiotics, such as antibiotic resistance and the presence of antibiotic residues in the final product, the European Commission banned the use of antibiotics as growth agents in 2006 [1]. This situation motivated many researchers to investigate and look for alternatives to antibiotics, and to find new substances that have positive effects both on the growth of chickens and feed utilization. By doing so the researchers also try to please consumers' increased demands for the usage of natural products as alternative additives in foods [2]. In that sense, research has been done on the use of probiotics, prebiotics, antioxidants, acidifiers, enzymes and various plant products as additives in broiler feeding and recently the propolis has also been considered as potential new additive [1, 3 - 5].

Propolis belongs to a group of natural substances of animal and vegetable origin with a particularly expressed antioxidant and antimicrobial properties [5, 6]. The bioactive components of propolis include flavonoids, phenolic acids 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 [5 - 7].

In recent years there has been several studies that investigated the effect of propolis on broilers production indicators however the results of these studies are not unambiguous and there are still no recommendations on the quantities of these substances that would result in the best production results [1, 5, 8 - 10]. Furthermore, several studies investigated the influence of propolis on biochemical profile of broilers blood but the results of these studies are also ambiguous [3, 11 - 13].

The aim of this study was to determine the effect of propolis supplementation on weight gain, feed conversion and selected biochemical blood parameters of broilers.

2. Materials 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. The experimental protocol was approved by the Committee for Animal welfare of the Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek (Approval code: 602-04/19-01/04; 2158-94-02-19-05).

2.1 Materials

The study included a total of 180 day-old chickens of the Ross 308 provenance. The fattening trial of the chickens was carried out on a family farm in Eastern Croatia under the supervision of the Division for Animal Production and Biotechnology, Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek. Total of 180 chickens of the Ross 308 provenance, evenly distributed sexes, were randomly divided into 3 groups (60 chickens in each group), one of which was the control group (C) and the other two experimental groups (E1 and E2). 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 [14]. Fattening was conducted on the wooden sawdust, and lasted for 6 weeks (42 days). During the study, feed and water were given to chickens' *ad libitum*. From days 1 - 21 of the study chickens were fed a mixture of starter, and from days 22 - 42 of the study they were fed a finisher mixture. Throughout the study the control group (C) of chickens was fed a standard feed mixture without additives, while the experimental groups of chickens (E1 and E2) were fed feed mixtures containing propolis as follows: E1 group: feed mixture + 2 g of propolis/kg of feed mixture; E2 group: feed mixture + 4 g of propolis/kg of feed mixture. Blending of propolis into the feed mixture was done in a vertical mixer (Briketstroj Ltd., Valpovo, Croatia).

2.2 Methods

Individual body weight (BW) of each broiler was measured on 1, 7, 14, 21, 28, 35, and 42 day of the feeding trial using an electronic scale Avery Berkel FX 220. Based on the measured values the average value of body weight of broilers from all the groups has been calculated, while difference between body weights was used for the calculation of weight gains (WG). During the trial, feed consumption (FC) was recorded in weekly intervals for each group of broilers. Based on the total amount of consumed feed and overall weight gain, feed conversion ratio (FCR) was calculated for each week.

Blood sampling was performed on day 42 of the study, on randomly selected chickens (14 birds from each group). 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, HDL cholesterol, LDL 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); HDL cholesterol (HDL, mmol/L); LDL cholesterol (LDL, 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.

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. On all statistical analyses, two-sided p-values of 0.05 and lesser ones were considered significant. Statistical analysis was done using statistical package Statistica for Windows 2010 (version 10.0, StatSoft Inc., Tulsa, OK).

3. Results and Discussion

The average values of the calculated weight gains of broilers from all the groups by the feeding period are shown in Table 1. Statistical analysis has shown that there were no statistically significant differences in average weight gain of broilers between the experimental groups and the control group on the 1 (p = 0.547), 2 (p = 0.464), 3 (p = 0.792), 4 (p = 0.426), 5 (p = 0.925) and 6 (p = 0.971) week of the feeding trial.

These results are opposite to the results of the study by Klaric *et al.*, Abou-Zeid *et al.*, Prakatur *et al.*, Tekeli *et al.*, Seven *et al.*, Khojasteh and Shivazad, and Shaddel-Tili *et al.*, [1, 4, 5, 11, 15 - 17], who have all determined positive effect of propolis on weight gain of broilers. However, the results of this study are consistent with the results of the study by Kleczek *et al.*, and Gheisari *et al.*, who found that there was no statistically significant difference in weight gain between experimental groups of chickens fed with the addition of propolis and control group of chickens [10, 18]. Furthermore, study by Abbas found out that there was statistically significant difference in weight gain between chickens fed with the addition of 0.5 and 1.5 g of propolis per kg of feed and control group of chickens while there was no statistically significant difference in weight gain between chickens fed with the addition of 2.5 g of propolis per kg of feed and control group of chickens [19]. The latter result is in concordance with the results of this study.

The values of weekly feed conversion for all broiler groups are shown in Figure 1. Statistical analysis has shown that there was no statistically significant difference in values of weekly feed conversion between the experimental groups and the control group of chickens on any week of the feeding trial (p = 0.368).

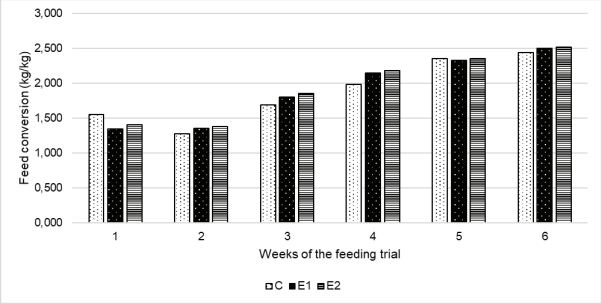

Figure 1. Feed conversion for all broiler groups according to the week of the feeding trial

(Kruskal-Wallis test; p = 0.368)

C = control group; E1 = feed mixture + 2 g of propolis/kg of feed mixture; E2 = feed mixture + 4 g of propolis/kg of feed mixture

Table 1. Weekly weight gains of broilers (g)

Weeks	Group of broilers $\bar{x} \pm s$			p
	C	E1	E2	
1st	107.58 ± 13.93	110.25 ± 13.29	107.98 ± 14.95	**0.547
2nd	246.55 ± 49.22	237.28 ± 44.06	246.43 ± 47.31	*0.464
3rd	365.28 ± 106.37	352.80 ± 109.52	354.45 ± 110.32	*0.792
4th	460.52 ± 179.66	417.82 ± 197.53	433.10 ± 164.26	*0.426
5th	489.72 ± 222.36	496.62 ± 275.52	476.30 ± 268.34	**0.925
6th	597.70 ± 333.56	599.07 ± 394.93	584.88 ± 337.18	*0.971

Legend: *ANOVA; **Kruskal-Wallis test. $\bar{x}$ = mean; s = standard deviation; C = control group; E1= feed mixture + 2.00 g of propolis/kg of feed mixture; E2 = feed mixture + 4.00 g of propolis/kg of feed mixture.

When analyzing feed conversion ratio according to the group of broilers the results of this study are consistent with the results of the study by Prakatur *et al.*, and Angelovicova *et al.*, [5, 20], and are opposite to the results of the study by Tekeli *et al.*, and Seven *et al.*, [11, 15].

The values of the investigated biochemical parameters in chickens' blood on the 42 day of the fattening period according to the particular group of chickens are shown in Table 2. The study revealed that there were statistically significant differences in Na levels ($p = 0.039$) and Ca levels ($p = 0.045$), while there were no statistically significant differences in HDL cholesterol ($p = 0.719$), LDL cholesterol ($p = 0.382$), cholesterol ($p = 0.462$), triglycerides ($p = 0.481$), total proteins ($p = 0.249$), albumins ($p = 0.661$), globulins ($p = 0.058$), glucose ($p = 0.179$), Fe ($p = 0.068$), Mg ($p = 0.557$), K ($p = 0.590$), P ($p = 0.397$), and Cl ($p = 0.128$) levels between the control group and experimental groups.

The present study showed that there were statistically significant differences in Na and Ca levels between experimental groups and control group of chickens while there were no statistically significant differences in any of other investigated biochemical parameters on the 42 day of fattening. Considering the values of blood glucose, this study showed that there were no statistically significant differences between the experimental groups and control group of chickens which is in concordance with the results of the study done by Tekeli *et al.*, [11]. In their study, Klaric *et al.*, showed that there was a statistically significant difference in blood values of glucose, cholesterol, total proteins, globulins, albumins, Na and K while there was no statistically significant difference in values of triglycerides, Ca, P, Mg, Fe and Cl between the groups of chickens on the 42 day of the fattening period which is partially consistent with the results of present study [13]. The results of this study are also partially consistent with the results of the study done by Petruska *et al.*, who reported that chickens fed with the addition of propolis had on the42nd day of fattening a significantly lower level of P and Mg 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 Na, K, Cl and Ca in the blood of chickens [21]. In the study done by Attia *et al.*, [3], the authors had found that chickens in the experimental group fed with the addition of propolis and/or bee pollen had on the 35 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 present study that did not determine such differences. Like the present study, the study done by Gheisari *et al.*, also did not find the statistically significant differences between the experimental groups and control group of chickens in blood values of cholesterol, HDL cholesterol, LDL cholesterol and triglycerides [18]. In study done by Abbas it was showed that chicks fed 2.5 g/kg propolis had statistically significant reduction in total serum protein and albumin while increases in cholesterol and glucose levels as compared with the control group which was not confirmed in the present study [19].

All results of the present study points to the conclusion that there is a limit to which the addition of propolis has a positive effect on production performance and that the use of propolis in quantities above that limit will no longer have a positive effect on weight gain and growth of chickens. Bearing in mind that propolis

Table 2. Biochemical parameters in chickens' blood on the 42 day of the fattening period

Parameters	Group of chickens $\bar{x} \pm s$			p
	C	E1	E2	
GLUC (mmol/L)	14.02 ± 0.80	14.43 ± 0.75	14.51 ± 0.61	*0.179
CHOL (mmol/L)	2.90 ± 0.49	3.00 ± 0.33	2.82 ± 0.28	*0.462
TRG (mmol/L)	0.71 ± 0.22	0.79 ± 0.14	0.72 ± 0.17	*0.481
HDL (mmol/L)	2.03 ± 0.25	2.09 ± 0.21	2.02 ± 0.19	*0.719
LDL (mmol/L)	0.55 ± 0.22	0.55 ± 0.16	0.47 ± 0.13	**0.382
TP (g/L)	26.54 ± 3.32	25.20 ± 1.82	24.67 ± 2.09	**0.249
GLO (g/L)	16.77 ± 2.40	15.76 ± 1.08	15.15 ± 1.33	**0.058
ALB (g/L)	9.76 ± 1.04	9.44 ± 0.98	9.52 ± 0.94	*0.661
Fe (µmol/L)	13.06 ± 2.17	14.59 ± 1.50	12.74 ± 2.68	*0.068
Ca (mmol/L)	2.54 ± 0.09	2.50 ± 0.07	2.46 ± 0.09	*0.045
Na (mmol/L)	156.14 ± 2.68	154.29 ± 1.64	153.50 ± 2.88	**0.039
P (mmol/L)	2.14 ± 0.31	2.05 ± 0.13	2.03 ± 0.18	*0.397
Mg (mmol/L)	0.88 ± 0.05	0.90 ± 0.04	0.88 ± 0.05	*0.557
K (mmol/L)	6.01 ± 0.74	6.06 ± 0.39	5.86 ± 0.39	*0.590
Cl (mmol/L)	113.64 ± 2.17	113.14 ± 1.29	114.64 ± 1.69	**0.128

Legend: *ANOVA; **Kruskal-Wallis test. $\bar{x}$ = mean; s = standard deviation; C = control group; E1= feed mixture + 2.00 g of propolis/kg of feed mixture; E2 = feed mixture + 4.00 g of propolis/kg of feed mixture.

is rich in antioxidant substances such as flavonoids, phenols and its derivatives it is possible that extremely large amount of these substances lead to the side effects that are manifested themselves in the absence of positive influence of added propolis (in amounts of 2 and 4 g per kg of feed) on production parameters of chickens, as it had been showed in research with royal jelly on other animal species [22, 23]. The mechanism of this side effect is based on the fact that uncontrollably high intake of antioxidants disrupts the redox balance between processes of oxidation and reduction and induces reductive stress that is, changing the body's reduction levels into an extremely reduced state, which can cause serious changes in cellular functions and lead to pathological mechanisms to the same way as well as increased oxidative stress [24].

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4. Conclusions

- Following the results of this study it can be concluded that applied doses of propolis did not affect the observed performance indicators and selected biochemical blood parameters of broilers thus it is important to evaluate the effects of such supplementation through some other indicators.

- Further studies are needed to better understand underlying mechanisms which determined the effects of propolis supplementation on performance indicators and biochemical blood parameters in broiler chickens.

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