

THE INFLUENCE OF PROPOLIS AS SUPPLEMENT DIET ON BROILER MEAT GROWTH PERFORMANCE, CARCASS BODY WEIGHT, CHEMICAL COMPOSITION AND LIPID OXIDATION STABILITY

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Abstract

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The present experiment was aimed to study the effect of the propolis extract as supplement diet on the broiler chickens growth performance, breast and thigh weights, meat chemical composition and lipid oxidation stability. A total of 120 chicks in one day old, which were divided into 4 groups (n = 30) for 42 days. To the experimental groups were added propolis extract in doses of 200 mg.kg⁻¹ (II), 300 mg.kg⁻¹ (III) and 400 mg.kg⁻¹ (IV). At the end of the experiment the results were shown that the body weight gain after 21 and 42 days has been increased and there were found significant differences (P ≤ 0.05) between control and experimental groups also the feed intake has been increased and there were (P ≤ 0.05). The FCR was higher in the control group. The carcass body weight breast and thigh weights were increased and there were no significant differences while, the abdominal fat and liver weights were decreased. No significant difference was occurred on chemical composition of breast and thigh muscles, whereas the muscle moisture tends to increase and fat content and energy value were decreased. Interestingly, the lipid oxidation stability measured as TBARS during the freezing storage for 6 months has been decreased malondialdehyde (MDA) in the experimental groups and there were found significant (P ≤ 0.05) in the breast muscles between control group and experimental groups. From the present study were concluded after administration the propolis extract that broiler growth performance has been increased and the lipid oxidation (MDA) during the freezing storage (–18 °C) have been decreased.

Keywords: propolis, broiler, growth performance, chemical composition, lipid oxidation

INTRODUCTION

Poultry meat represents an important component of human diet. Complete feed mixtures for broiler chickens are often enriched with various additives

as vegetable oils, probiotic, prebiotic and enzyme preparations (Lee *et al.*, 2003, 2004; Shalmany and Shivazad, 2006). The importance of these alternative supplement consists mainly in the replacement of

antibiotics and coccidiostats which were banned by the European Union since 2006 for poultry husbandry. Recently, the bee products (pollen, propolis and its extracts) using as an antibiotic alternative feed additive in broiler diets, which ultimately may favourable affected the health, performance of poultry, economic use of feed mixtures for poultry and overall economics of the poultry industry (Prytyk *et al.*, 2003; Wang *et al.*, 2004; Shalmany and Shivazad, 2006; Seven *et al.*, 2008). In general propolis is a glue-like natural resinous substances that honey bees collected from a large variety of plant seedling and buds, which is used in folk medicine of at least 300 years b. c. (Ghisalberti, 1979). Propolis is a mixture of waxes, sugars and plant exudates and its chemical composition in different samples includes more than 300 compounds, so its biological properties are diverse and its composition is directly related to that of the bud exudates collected by bees (Ghisalberti, 1979; Markham *et al.*, 1996; Bankova *et al.*, 2000; Banskota *et al.*, 2001), in the animal nutrition the propolis has been added in grains or extract form into animals feed mixture as supplemental diet as well as antibiotic (Ghisalberti, 1979; Pepelnjak *et al.*, 1985; Velikova *et al.*, 2000), antifungal (Dimov

et al., 1991; Murad *et al.*, 2002), antiviral (Amoros *et al.*, 1994), local anesthetic (Paintz and Metzner, 1979), anti inflammatory (Strehl *et al.*, 1993; Miyataka *et al.*, 1997), antioxidant (Sun *et al.*, 2000; Isla *et al.*, 2001), hepatoprotective (Gonzales *et al.*, 1995), immune stimulating (Dimov *et al.*, 1991), and cytostatic (Frenkel *et al.*, 1993; Banskota *et al.*, 2001). Prytyk *et al.* (2003) and Wang *et al.* (2004) were found that the propolis has a positive effect on feed intake, body weight aslo they that found the propolis content flavonoid which led to improvement meat antioxidant. The biological properties of propolis are based on some contents such as, flavonoid, phenolic acid, terpenoid, caffeic acid and their related esters contents, but the percentage of this content are depended to the collecting time, location, and plant source (Greenaway *et al.*, 1991; Markham *et al.*, 1996). Propolis can be used as bio preserving in the process of preparing and food (Erkmen and Ozcan, 2008) or pharmaceutical products storage (Pavilonis *et al.*, 2008). In the present study, we aimed to evaluate the effects of the propolis extract on growth performance organs weights, meat chemical composition and oxidative stability of the broiler chicken meat during storage.

I: Composition of the broiler feed mixture

Ingredients (%)	Starter HYD-01 (1 to 21 days of age)	Grower HYD-01 (22 to 42 days of age)
Wheat	35.00	35.00
Maize	35.00	40.00
Soybean meal (48% N)	21.30	18.70
Fish meal (71% N)	3.80	2.00
Dried blood	1.25	1.25
Ground limestone	1.00	1.05
Monocalcium phosphate	1.00	0.70
Fodder salt	0.10	0.15
Sodium bicarbonate	0.15	0.20
Lysine	0.05	0.07
Methionine	0.15	0.22
Palm kernel oil Bergafat	0.70	0.16
¹ Premix Euromix BR 0,5 %1	0.50	0.50
Nutrient composition (g.kg ⁻¹)		
Crude protein	210.76	190.42
Fibre	30.19	29.93
Ash	24.24	19.94
Ca	8.16	7.28
P	6.76	5.71
Mg	1.41	1.36
ME (MJ/kg)	12.02	12.03

¹ active substances per kilogram of premix: vitamin A 2 500 000 IU; vitamin E 50 000 mg; vitamin D3 800 000 IU; niacin 12 000 mg; d-pantothenic acid 3 000 mg; riboflavin 1 800 mg; pyridoxine 1 200 mg; thiamine 600 mg; menadione 800 mg; ascorbic acid 50 000 mg; folic acid 400 mg; biotin 40 mg; vitamin B12 10.0 mg; choline 100 000 mg; betaine 50 000 mg; Mn 20 000 mg; Zn 16 000 mg; Fe 14 000 mg; Cu 2 400 mg; Co 80 mg; I 200 mg; Se 50 mg

MATERIAL AND METHODS

Animals and Diets

The experiment was carried out in the test poultry station of Slovak University of Agriculture in Nitra. The experiment included 120 one day-old chicken hybrid combination Ross 308, which were divided into 4 groups ($n = 30$): control (I) and three experimental (II, III, IV) groups. Chickens experimental diets were fed from one day old to 42 days of age by *ad libitum* system with feed mixture HYD-01 (until the age of 21st days) and HYD-02 (from 22nd to 42nd days of age). Feed mixtures HYD-01 and HYD-02 were produced without any antibiotic preparations and coccidiostats. Their nutritional value (Tab. I) was the same in each group during the whole experiment and to the experimental group was added propolis extract indoses of 200 mg.kg⁻¹ (II), 300 mg.kg⁻¹ (III) and 400 mg.kg⁻¹ (IV).

Propolis Preparation

Propolis samples were collected from Slovak republic, cut into small pieces and extracted for one hour with 80% ethanol at the temperature of 80 °C to obtain the extract (Krell, 1996). After centrifugation, the extract was evaporated by using a vacuum evaporator at 40–50 °C. To obtain the desired concentrations of propolis in feed mixture the 20, 30 and 40 g of propolis residue were dissolved at 1000 cm³ of 80% ethanol. Each 1000 cm³ of propolis extracts were used per 100 kg of feed mixture.

Sample Analysis

At the end of the fattening period the chickens has been slaughtered for analysis. The economic feed utilization (feed intake per fattening period, feed conversion), organs (carcass weight, weights of live body, breast and thigh muscle, liver and abdominal fat) were evaluated in this study. For the determination the chemical composition the meat samples have been taken from the breast and thigh muscles. Chemical composition of broiler chickens meat (content of moisture, fat and protein) was carried out by INFRA TEC 1265 Meat Analyzer (FOSS, Sweden) device. The energy values were calculated with conversion factors for fat and

protein was evaluated according to Strmiska *et al.* (1988) methods. For the determination of the lipid changes, the mixed samples of the breast and thigh muscle have been packed in polyethylene bags and stored for 6 months at –18 °C. Lipid oxidation was assessed by the 2-thiobarbituric acid (TBA) test following the recommendations of (Marcinčák *et al.*, 2004) and measured by spectrophotometric method at 532 nm by UV-VIS spectrophotometer T80 (PG Instruments, Great Britain). TBARS values were calculated from a standard curve of malondialdehyde (MDA) and expressed as MDA mg.kg⁻¹ of the sample.

Statistical Analysis

Data for this experiment were evaluated by using General Liner model using SPSS Statistics 17.0 (Statistical Packages for the Social Sciences, released 23 August 2008). Significant differences were determined by One-Way ANOVA followed by Duncan test ($P \leq 0.05$) was set as the limit of significance.

RESULTS AND DISCUSSION

Data presented in Tab. II shows the effects of the propolis as a dietary supplement on broiler chickens growth performance. Either feeding propolis tended to increase body weight gain and feed intake. Consequently, feed conversion ratio was improved by feeding propolis. We found that the broiler body weight gain in 21, 42 days after propolis administration in the experimental groups were higher compared to the control group and there were significant differences ($P \leq 0.05$) this result is in agreement with (Shalmany and Shivazad, 2006; Seven *et al.*, 2008 and Tekeli *et al.*, 2010) who found that the propolis has a positive effect on the broiler body weight gain. The highest values of the feed intake were found in the experimental group II (3857.18 ± 31.36 g) and the lowest feed intake value was found in the control group (3754.43 ± 9.25 g) and were found significant differences ($P \leq 0.05$) between control group and experimental group. We found that various amounts of propolis extract increase ($P \leq 0.05$) of the feed intake compared to the and the control group, this result confirms the results of Shalmany and Shivazad (2006) who

II: Effect of the propolis supplementation on growth performance in broilers

Item	I. Control (no propolis)	II. (propolis 200 mg.kg ⁻¹)	III. (propolis 300 mg.kg ⁻¹)	IV. (propolis 400 mg.kg ⁻¹)
Initial BW (g)	42.10 ± 3.70	43.00 ± 2.71	43.80 ± 3.12	43.30 ± 4.30
BWG g/21 days	604 ± 18.88 ^a	726.67 ± 18.48 ^b	760.67 ± 27.48 ^b	798.00 ± 17.69 ^b
BWG g/42 days	2155.00 ± 23.39 ^a	2363.33 ± 44.85 ^b	2462.33 ± 48.55 ^b	2315.33 ± 66.16 ^b
FI g/42 days	3754.43 ± 9.25 ^a	3857.18 ± 31.36 ^b	3834.91 ± 17.66 ^b	3826.43 ± 17.66 ^b
FCR	1.74 ± 0.024 ^b	1.63 ± 0.03 ^{ab}	1.56 ± 0.03 ^a	1.66 ± 0.05 ^{ab}

^{a,b}Values are expressed as means ± standard error ($n = 30$); Data were analysed by two-way analysis of variance and Duncan multiple tests. Means with different superscripts differ from each other significantly; BW: body weight; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio

III: Effect of the propolis supplementation on the organ weights in broilers

Item	I. Control	II.	III.	IV.
	(no propolis)	(propolis 200 mg.kg ⁻¹)	(propolis 300 mg.kg ⁻¹)	(propolis 400 mg.kg ⁻¹)
Carcass (g.100g ⁻¹ BW)	72 ± 1.33	75 ± 1.75	76 ± 2.66	73 ± 1.83
Breast (g.100g ⁻¹ BW)	12.15 ± 0.44	12.88 ± 0.39	12.45 ± 1.13	12.33 ± 0.39
Thigh (g.100g ⁻¹ BW)	9.83 ± 0.37 ^a	10.63 ± 0.50 ^{ab}	11.33 ± 0.47 ^b	10.67 ± 0.46 ^{ab}
Liver (g.100g ⁻¹ BW)	1.90 ± 0.06	1.85 ± 0.10	1.85 ± 0.08	1.87 ± 0.10
Abdominal fat (g.100g ⁻¹ BW)	2.05 ± 0.08	1.87 ± 0.16	2.03 ± 0.11	2.02 ± 2.02

^{a,b}Values are expressed as means ± standard error (n = 30); Data were analysed by two-way analysis of variance and Duncan multiple tests; Means with different superscripts differ from each other significantly; BW: bodyweight; I, II, III and IV: experiment groups.

used propolis to feed broiler and they were found that the feed intake was increased, also our study in agreement with Kamel *et al.* (2007) who tested propolis on rabbits they were found that the feed intake was increased. This positive effect can be probably attributed to quicker digestion and passage of the nutrients through the digestive system which will have been emptied earlier and feed intake will have been promoted. Due to the active ingredients of this natural product, the formation of more stable intestinal flora digestive system is a consequence of better digestion (Tekeli *et al.*, 2011). Also, (Ghisalberti, 1979) reported the propolis has improved the broiler feed intake, this is due because the propolis content high amount of the flavonoids. The present findings show that the feed conversion ratio (FCR) in the experimental groups was improved compared to the control group, this results in agreement with (Botsoglou *et al.*, 2004; Seven *et al.*, 2008; Pourali *et al.*, 2010; Tekeli *et al.*, 2011; Daneshmand *et al.*, 2012) who was found that the propolis increased the (FCR) on broiler chickens. The reason which led propolis to the improve the growth performance on the broiler because, the propolis has improve the digestive utilization of iron and the regeneration efficiency of haemoglobin, especially during recovery from an anaemic syndrome, on other hand propolis has a positive effect on phosphocalcic metabolism and maintain an appropriate level of magnesium metabolism and improvement in the digestive utilization of these minerals (Haro *et al.*, 2000).

Tab. III shows that the carcass weight, breast and thigh weights were higher in the experimental groups compared to control group and there no significant differences. This result confirms the study of (Denli *et al.*, 2005; Seven *et al.*, 2008) who found that the propolis increased the broiler's organ weights, moreover the propolis was decrease the liver and abdominal fat weight in experimental groups and these results are in agreement with (Ghisalberti *et al.*, 1979; Denli *et al.*, 2005) who used propolis as supplement dietary for broiler, also the present result is not agree with (Seven *et al.*, 2008) who were used propolis and they found the abdominal fat was increased in the experimental groups except the group which was fed with basal diet supplemented with 3 g of propolis. The reason

that makes propolis reduces the abdominal fat in broiler because the propolis content flavonoids, and the flavonoids are decrease plasma lipid levels, improve glucose tolerance, and attenuate obesity. One possible mechanism underlying these physiological effects is reduction of hepatic levels of the mRNA for stearoyl-CoA desaturase-1 (SCD1), since repression of this enzyme reduces hyperlipidaemia and adiposity (La Nita *et al.*, 2011).

Chemical composition of chicken meat has been often different (Horniaková *et al.*, 1999), particularly influenced by nutrition, application of new trends in nutrition, breeding environment and a relatively large impact. However, Tab. IV shows us the results of the breast and thigh muscles chemical composition, where they found that the moisture content was higher in the experimental groups compared to the control groups and there were no significant differences ($P \geq 0.05$). Further, the present findings is supports (Seven *et al.*, 2008) who was found that the moisture was higher in the experimental groups, also with (Čuboň *et al.*, 2013; Haščík *et al.*, 2013) tested bee pollen for broiler and found similar results. Protein and fat from the nutritional point of view constitute a significant part of broilers muscles (Duclos *et al.*, 2007; Berri *et al.*, 2008). Further, the broiler breast and thigh protein content have shown in the Tab. IV after administration propolis in where that the protein content has been was decreased in all experimental groups compared to control group. The recent study has support (Haščík *et al.*, 2013) who was evaluated the chemical composition of broiler muscles after addition bee pollen into their feed mixture. Fat constitutes an energy reservoir of the broiler chickens also fat content vitamins which soluble in fat, daintiness (Ševčíková *et al.*, 2006, 2008) as well as supplier of essential fatty acid which can be influenced by nutrition (Zelenka *et al.*, 2008), although dietary factors are suspected to be important determinants of (CHD) coronary heart disease (Gary *et al.*, 1992). Our result shows the breast and thigh fat content in control was higher compared to experimental groups and in the breast there were significant differences ($P \leq 0.05$) between control group and (III, IV) groups. This result in agreement with (Čuboň

IV: Chemical composition of the breast and thigh muscles after feeding broilers on different levels of Slovak propolis

Item	I. Control	II.	III.	IV.
	(no propolis)	(propolis 200 mg.kg ⁻¹)	(propolis 300 mg.kg ⁻¹)	(propolis 400 mg.kg ⁻¹)
Breast muscle				
Moisture (g.100g ⁻¹)	73.97 ± 0.19	74.17 ± 0.15	74.03 ± 0.14	74.18 ± 0.11
Crude protein (g.100g ⁻¹)	23.96 ± 0.19	23.42 ± 0.22	23.38 ± 0.14	23.75 ± 0.13
Crude fat (g.100g ⁻¹)	1.59 ± 0.09 ^b	1.41 ± 0.13 ^b	1.07 ± 0.14 ^a	1.07 ± 0.08 ^a
Energy value (kJ.100g ⁻¹)	451.52 ± 3.40 ^b	445.41 ± 3.28 ^{ab}	441.65 ± 4.85 ^{ab}	438.13 ± 2.60 ^a
Thigh muscle				
Moisture (g.100g ⁻¹)	68.49 ± 0.80	69.48 ± 0.51	68.41 ± 0.33	69.25 ± 0.48
Crude protein (g.100g ⁻¹)	18.98 ± 0.23	18.97 ± 0.18	19.03 ± 0.14	18.78 ± 0.13
Crude fat (g.100g ⁻¹)	11.56 ± 0.97	10.74 ± 0.64	11.50 ± 0.46	10.97 ± 0.49
Energy value (kJ.100g ⁻¹)	752.36 ± 33.76	722.8 ± 22.50	754.32 ± 15.12	727.94 ± 18.21

^{a,b}Values are expressed as means ± standard error (n = 10); Data were analysed by two-way analysis of variance and *Duncan* multiple tests. Means with different superscripts differ from each other significantly; I, II, III and IV: experiment groups. I, II, III and IV: experiment groups.

V: Effect of storage in freeze (-18 °C) on the concentration of oxidative (malondialdehyde) (MDA mg.kg⁻¹) in breast and thigh muscle after feeding broilers on different levels of propolis

Time of storage	I. Control	II.	III.	IV.
	(no propolis)	(propolis 200 mg.kg ⁻¹)	(propolis 300 mg.kg ⁻¹)	(propolis 400 mg.kg ⁻¹)
Beast muscle				
Day – 1	0.074 ± 0.002	0.052 ± 0.007	0.040 ± 0.005	0.051 ± 0.004
Month – 1	0.080 ± 0.003 ^c	0.069 ± 0.003 ^{ab}	0.046 ± 0.008 ^a	0.053 ± 0.006 ^{ab}
Month – 2	0.084 ± 0.003 ^b	0.066 ± 0.004 ^a	0.052 ± 0.007 ^a	0.057 ± 0.006 ^a
Month – 3	0.088 ± 0.002 ^b	0.074 ± 0.003 ^a	0.065 ± 0.004 ^a	0.067 ± 0.003 ^a
Month – 4	0.105 ± 0.008 ^b	0.078 ± 0.001 ^a	0.074 ± 0.006 ^a	0.078 ± 0.011 ^a
Month – 5	0.127 ± 0.006 ^b	0.096 ± 0.010 ^a	0.087 ± 0.006 ^a	0.089 ± 0.005 ^a
Month – 6	0.142 ± 0.010 ^b	0.122 ± 0.002 ^{ab}	0.110 ± 0.006 ^a	0.100 ± 0.005 ^a
Thigh muscle				
Day – 1	0.110 ± 0.013 ^b	0.086 ± 0.005 ^b	0.040 ± 0.003 ^a	0.082 ± 0.015 ^b
Month – 1	0.107 ± 0.016	0.084 ± 0.013	0.080 ± 0.008	0.077 ± 0.009
Month – 2	0.107 ± 0.006 ^b	0.094 ± 0.008 ^{ab}	0.084 ± 0.008 ^{ab}	0.0080 ± 0.006 ^a
Month – 3	0.111 ± 0.008	0.096 ± 0.012	0.089 ± 0.006	0.094 ± 0.008
Month – 4	0.120 ± 0.008 ^b	0.110 ± 0.008 ^{ab}	0.103 ± 0.006 ^{ab}	0.091 ± 0.004 ^a
Month – 5	0.133 ± 0.009 ^b	0.113 ± 0.009 ^{ab}	0.106 ± 0.010 ^{ab}	0.095 ± 0.002 ^a
Month – 6	0.142 ± 0.010 ^b	0.122 ± 0.002 ^{ab}	0.110 ± 0.006 ^a	0.100 ± 0.005 ^a

^{a,b}Values are expressed as means ± standard error (n = 10); Data were analysed by two-way analysis of variance and *Duncan* multiple tests. Means with different superscripts differ from each other significantly; I, II, III and IV: experiment groups.

et al., 2013; Haščík *et al.*, 2013) who was added bee pollen into the broiler's feed mixture.

Tab. V summarizes the findings of application of propolis extracts (200, 300 and 400 mg.kg⁻¹) in broiler chickens nutrition, where were found that the propolis extrect has a positive affected on broiler lipid oxidation stability as TBARS on breast and thigh muscles immediately after 24 hours of *post mortem* during six months of freezing storage which led to decrease malondialdehyde (MDA) in experimtal groups compared the control group and highest value of malondialdehyde (MDA) were found in control groups (0.142 ± 0.010 MDA mg.kg⁻¹) after 6 months in the breast and thigh

muscles. Moreover, in breast muscle after (1, 2, 3, 4, 5 and 6 months) were found significant differences ($P \leq 0.05$) between control group and experimental groups, also in the thigh muscles were found significant differences ($P \leq 0.05$) in first day between control and III group also after 2 months, 4 months, and 5 months were found ($P \leq 0.05$) between control and IV group, similar after 6 months were found ($P \leq 0.05$) between control and groups III, IV. However, our study confirms by (Basnet *et al.*, 1997; Banskota *et al.*, 2000; Seven *et al.*, 2010) who tested propolis for meat oxidative stability and there were found that the propolis has a positive effect on broiler meat which led to decrease the MDA

in the meat. Also the obtained results of lipid oxidative stability in cooling and freezing conditions approve in agreement with (Sahin *et al.*, 2002; Young *et al.*, 2003; Kennedy *et al.*, 2005; Imik *et al.*, 2010), who suggested that lipid oxidation stability of broiler chickens fat during its cooling and freezing storage decrease. The reason why propolis prevents lipid oxidation in broiler meat because propolis content chyrisin as one of the propolis compounds which having hepatoprotective and antioxidant activities (Sathivelu *et al.*, 2009) also benzoic acid derivative

exhibits antioxidant effects using inhibition assays of luminal luminescence, 2,2-diphenyl-1-picrylhydrazyl and lipoperoxidation, particularly caffeic acid, caffeoylquinic acid and cinnamic acid are effective O_2 – scavenging activity (Christov *et al.*, 2006; Nakajima *et al.*, 2007). Besides, flavonoids inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility, and the activity of enzyme systems, including cyclooxygenase (COX) and lipoxygenase (Havsteen, 2002).

CONCLUSION

From the present study, we found that the propolis has a positive effect on the broiler growth performance which led to increases broiler body gain and feed intake and while FCR was decreased. On the other hand the broiler carcass body weight, breast and thigh were increasing, whereas the liver and abdominal fat were decreasing. Moreover, the propolis improves broiler lipid oxidation stability in freezing, storage (-18°C) as measured by TBARS during the study period (6 months) which were found that the MDA was decreased in the breast and thigh muscles.

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