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Effects of Dietary Propolis Supplementation on Performance and Egg Quality in Laying Hens

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Abstract: This experiment was conducted to evaluate the efficacy of supplemental propolis on the performance and egg quality of laying hens. Two hundred and forty, 18 weeks old Bovans White strain laying hens were divided into four groups of 60 each. They were fed 0 (P-0), 100 (P-1), 200 (P-2) and 400 (P-4) mg kg^{-1} of supplemental propolis for 32 weeks. Feed and water were supplied *ad libitum*. The live weight of the animals was weighed before and at the end of the experiment whilst feed consumption, feed conversation ratio, egg production, egg weight, mortality, Haugh unit and shell thickness were determined at 28 days intervals throughout the experiment. At the end of the study, it was determined that the live weight of laying hens fed on a diet supplemented with 400 mg kg^{-1} propolis had increased ($p<0.05$). However, propolis doses used in this study did not have significant dietary effects on either performance criteria including feed consumption, feed conversation ratio, egg production, egg weight, mortality or egg quality criteria including Haugh unit and shell thickness ($p>0.05$). In conclusion, as the results of the present study demonstrated that in laying hens, supplementation of the ration with propolis did not induce any adverse effect on performance, egg quality or survival rate, it is suggested that various doses of propolis could be used in egg production.

Key words: Laying hen, propolis, performance, egg quality, group

INTRODUCTION

In poultry breeding, the main factors which affect performance, include temperature, humidity, stocking density, lighting, management-feeding, diseases and pests. Of the listed factors, feed and feedstuffs in the composition of the ration bear great significance. In order to increase the productivity of poultry not only should growth rate and feed conversion ratio be improved but also animal health should be protected. For this reason, antibiotics, chemotherapeutics and synthetic hormones have been used as feed additives for many years with an aim to protect animal health and increase productivity. However, the use of, in particular antibiotics and chemotherapeutics as feed additives at low doses has led to the development of microbial resistance and the killing of not only pathogenic but also beneficial microorganisms. Furthermore, antibiotic use is subject to restrictions due to the risk posed by residues to human health. The restriction of the use of antibiotics, chemotherapeutics and synthetic hormones in rations has encouraged researchers to investigate alternative feed additives that would improve feed conversion ratios, protect animal health and reduce production costs, thereby, resulting in the conduct of multiple studies.

Propolis, proven to have several beneficial biological effects in recently conducted research is a natural product and has found use in poultry breeding. Propolis is a resinous mixture, consisting of resin collected by honeybees (*Apis mellifera* L.) from plant and tree buds and sprouts, later mixed with beeswax and the salivary secretions of these insects in the hive. A great many studies exist on the biological activities of propolis samples of different origin. To exemplify, Brazilian propolis has been shown to have antibacterial, cytotoxic, hepatoprotective and antioxidant effects (Orsi *et al*., 2005) whilst Bulgarian propolis has been determined to have bactericidal, antifungal and antiparasitic activities (Orsi *et al*., 2005; Velikova *et al*., 2000). To date, Turkish propolis has been ascertained to induce multiple biological effects of antibacterial (Silici *et al*., 2007), antifungal (Koc *et al*., 2005), antioxidant (Gulcin *et al*., 2010), anticarcinogenic (Eroglu *et al*., 2008) wound and burn healing (Han *et al*., 2005), immunomodulatory (Cetin *et al*., 2010) and food preservative (Sagdic *et al*., 2007) character.

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Only a limited number of studies are available on the use of propolis as an alternative feed additive, the first on its use in poultry rations dating back to 1976 (Bonomi *et al*., 1976). In research carried out in laying hens it has been reported that propolis improves live weight gain, egg production, egg weight and feed conversion ratios shows anabolic effect and induces the immune system (Bonomi *et al*., 1976; Giurgea *et al*., 1981a, b).

Supplemental propolis given to laying hens under heat stress has been shown to reduce mortality rates and increase feed consumption, live weight gain, feed digestibility, feed conversion ratios and egg shell thickness with no effect on egg quality and possible effect on heat stress-induced oxidative stress (Tatli-Seven, 2008). Furthermore, literature reports indicate that propolis has *in vitro* activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* infections and also suggest that bacteria known to be resistant to antibiotics such as benzyl/penicillin, tetracyclines and erythromycin are sensitive to propolis (Lotfy, 2006). Further reports indicate that propolis supplementation of broiler rations increases live weight gain by 20% (Ghisalberti, 1979), increases performance and decreases Lipid Peroxidation (LP) in broiler chickens under heat stress (Tatli-Seven *et al*., 2007), increases feed consumption and improves live weight gain and feed conversion ratios (Roodsari *et al*., 2004; Shalmany and Shivazad, 2006).

In view of propolis having been proven to induce several beneficial biological effects in studies conducted by various researchers, the present study was designed to investigate the effects of the supplementation of laying hen rations with propolis on performance and egg quality.

MATERIALS AND METHODS

Origin and chemical analysis of propolis: The propolis used in the present study was collected manually in September and October in 2008 from frames belonging to bee hives kept in a particular region of the Bunyan district of Kayseri Province in Turkey where poplar trees are grown intensively. The 30 g of the propolis in powder form was subjected to extraction after being mixed with 80% ethyl alcohol and stirred for 3 days in the dark on a magnetic stirrer. The resulting extract was filtered through Whatman-1 filter paper such that the filtering procedure was repeated three times. The extracts obtained were pooled and the alcohol content was vaporized in an evaporator (vacumed vaporizer) to produce pure propolis. The resulting pure propolis was preserved in a deep freezer (-18°C) until used. The chemical analysis of the propolis used in the trial was performed in accordance with the following procedure: 1 mg of propolis extract was reacted with 50 μL pyridine + 100 μL Bis (trimethylsilyl) Trifluoroacetamide (BSTFA) in a beaker containing 1% of Trimethylchlorosilane (TMCS) at 100°C for 30 min. Thereby, propolis was prepared for gas chromatography. The 1 mL of the sample was injected into a gas Chromatography-Mass Spectrometry (GC-MS) instrument to perform analysis. An Agillent Gas Chromatograph 6890 (GC), 5973 Mass Spectrometer (MS) and a capillary column of 30 m length, 0.25 mm id and 0.25 μm film thickness were used in the analyses. Helium was used as the carrier gas with a flow rate of 10 mL min^{-1}. For the analysis of propolis, the initial temperature of the column was set to 100°C (5 min) and was later raised to 150°C. Subsequently, the temperature was adjusted to 140°C for 2 min and increased to a final temperature of 280°C by increasing the temperature 2° at a time. The final temperature was maintained for a period of 60 min. The peak values determined in the analysis were identified by means of the reference library.

Animals, diets and management: The 18 weeks old 240 Bovans White strain laying hens constituted the material of the study. The animals were weighed individually for their allocation to groups comprising of animals with similar live weight ($p>0.05$). Accordingly, a control group and three trial groups each composed of 60 hens were established. Each of these four groups was further divided into 6 sub-groups of 10 hens which were housed in experimental units of a capacity of 5 hens. The animals were given *ad libitum* feed and water throughout the study. The hens housed in each experimental unit were fed in groups. Water was made continuously available by the use of nipple drinkers. A lighting period of 16 h including daylight was applied. In the present study, the control group (P-0) was fed on a basal ration whilst the trial groups were given basal rations supplemented with 100 (P-1), 200 (P-2) and 400 (P-3) mg kg^{-1} of propolis, respectively. The propolis doses given to the trial groups were solubilized in 70% ethanol and were added to the basal ration by means of pulverisation. The feed was mixed to ensure homogenous distribution and was prepared freshly at 15 days intervals. The chemical composition of the basal ration and its feedstuff constituents are shown in Table 1.

The amounts of nutrients in the basal ration were determined using the methods of analysis developed by AOAC International (AOAC, 1994) and Metabolisable Energy (ME) was also calculated as described by the National Research Council (NRC, 1984). The study was conducted for a period of 32 weeks between 6 November,

Table 1: Ingredients and chemical analyses of the basal diet given to the laying hens

Basal diets	Ingredients (%)
Maize	57.40
Full-fat soybean meal	9.00
Sunflower meal	1.45
Fish meal	2.00
Lime stone	8.43
MCP (Monocalcium Phosphate)	3.93
Vitamin-mineral premix[1]	0.25
Salt	0.25
DL-methionine	0.53
Chemical analysis (DM)	
Dry matter (%)	89.20
Metabolisable energy[2] (kcal kg^{-1})	2780.00
Crude protein (%)	17.04
Calcium (%)	3.80
Utilisable phosphorus (%)	0.42
Lysine (%)	0.80

[1]Vitamin and mineral premix provided per kg of diet: vitamin A: 12.500 IU; vitamin D: 3.000 IU; vitamin E: 30 mg; vitamin K_3: 4 mg; vitamin B_1: 3 mg; vitamin B_2: 7 mg; vitamin B_6: 5 mg; vitamin B_{12}: 20 μg; Niacin: 20 mg; Folic acid: 1 mg; Ca D-pantothenate: 15; D-Biotin: 0.05 mg; vitamin C: 50 mg; Colin chloride: 300 mg; Mn: 80 mg; Fe: 70 mg; Zn: 70 mg; Cu: 0.625 mg; Se: 0.188 mg; Co: 0.25 mg; Canthaxanthin: 1.5 mg; Apo-carotenoic acid: 0.5 mg. [2]Calculated. DM: Dry Matter

2008 and 18 June, 2009. Throughout the study, the lowest and highest temperatures of inside air and relative humidity values were recorded on a daily basis. The lowest and highest temperatures and relative humidity values measured were 12.3 and 24.0°C and 44, 74.4%, respectively.

Performance and egg quality measurements: In the present study, live weight (g) measurements were performed twice, firstly, prior to the start of the trial and secondly at the end of the trial. Furthermore, feed consumption (g), feed conversion ratios (kg feed/kg egg), egg production (%) (hen-day), Haugh unit, egg shell thickness (mm) (by the use of a digital calliper) and survival rates (%) were determined at 28 days intervals throughout the study.

The study was conducted in four groups including one control group and three trial groups using random complete design. The statistical analyses of the data, obtained at each stage of the study were performed using the SPSS 13.0 (Inc. Chicago, II. USA, 2004) Software by ANOVA in accordance with the General Linear Model. Differences between the mean values of the trial groups were compared by Duncan's multiple comparison test. Differences among means with $p<0.05$ were accepted as reperesenting statistically significant difference.

RESULTS AND DISCUSSION

Chemical composition of propolis: Analyses demonstrated that the chemical composition of propolis used in this study was made up of 37.83% of flavonoids such as pinocembrin, chrysin and galangin, 18.54% of organic acids and fatty acids including 9-octadecanoic acid and n-hexadecanoic acid, coumaric acid, octadecanoic acid, cinnamic acid and derivatives, 35.8% of aromatic acids and their esters, alcohols, aldehydes, ketones, terpenes and kinones, 4.89% of various hydrocarbons and 2.94% of other undefined components. The results of the chemical analyses performed suggest that the main constituents of the propolis used in the present study are phenolic compounds, organic acids and fatty acids, aromatic acids and their esters, alcohols, aldehydes, ketones, terpenes and kinones (Table 2).

Earlier conducted several researches have shown that in propolis samples collected from different parts of the world, biological activity arose from the different chemical compounds and compound groups found in the structure of propolis. It is known that the main sources of propolis in Europe and North America are poplar bud secretions. Similarly, recent studies have shown that the main source of Turkish propolis is also poplar trees (Popova *et al*., 2005). Chestnut (*Castanea* sp.), willow (*Salix* sp.) and eucalyptus (*Eucalyptus* sp.) trees constitute other sources of Turkish propolis (Sorkun *et al*., 2001). As poplar trees are endemic in continental climate zones and do not grow in tropical and subtropical climate zones, the plant origin and chemical structure of propolis originating from the latter completely differ from those of poplar propolis (Bankova, 2005).

Chemical analysis results of the present study have shown that Kayseri propolis is of the poplar type and displays features in agreement with findings previously reported by other researchers (Popova *et al*., 2005; Silici *et al*., 2007).

Performance and egg quality: The results of the statistical analyses of the data obtained in the present study are shown in Table 3. It was determined that differences between the mean initial body weights of the groups measured prior to the start of the trial were statistically insignificant ($p>0.05$) and that in-group values showed a homogenous distribution. On the other hand, differences between the mean final body weights of the groups measured at the end of the trial were statistically significant ($p<0.05$). The mean final body weights of the hens were determined as 1717.12±20.96, 1702.22±22.51, 1726.37±18.67 and 1769.22±19.11 g for the groups P-0, P-1, P-2 and P-3, respectively. These data demonstrated that the supplementation of the basal ration with 400 mg kg^{-1} of propolis led to an increase in live weight. Furthermore, of the performance parameters, the trial groups did not display any statistically significant difference for feed consumption, feed conversion ratio, egg production, egg

Table 2: Chemical composition of propolis assessed by GC-MS (gas chromatography mass spectrometry)

Compounds	RT (min)	TIC (%)
Phenolics		
4,5 dimethoxy-(2-propenyl) 2-phenol	27.93	1.25
Pinocembrin	31.06	14.75
5-methoxy-3,7-dihydroxyflavanone	32.50	1.57
5-hydroxy-7-methoxy flavone (tectochrysin)	32.72	5.27
7-dihydroxy-3- methoxyisoflavone	33.30	1.34
Chrysin	34.12	7.67
Galangin	34.84	4.90
4,5-dihydroxy-7-methoxyflavanone	35.24	1.08
Organic and fatty acids		
Decanoic acid	9.03	0.23
4-pentenoic acid	13.31	1.74
Cinnamic acid	20.30	0.29
3-hydroxy-4-methoxycinnamic acid	20.73	1.82
2-propenoic acid	16.74	2.70
3,4-dimethoxycinnamic acid	20.91	3.40
n-hexadecanoic acid	21.78	0.41
Coumaric acid	22.93	0.19
m-hydroxycinnamic acid	24.71	0.88
9-octadecanoic acid	25.12	2.05
Octadecanoic acid	25.49	0.21
1,3-benzenedicarboxylic acid	29.16	4.62
Alcohols, ketones and terpenes		
Nerolidol	13.55	0.39
Chrysophanol	33.91	7.49
5-3,3-dimethyl-cyclohexanone	34.38	1.36
Ethanone	8.18	0.21
3-methoxy acetophenone	8.50	0.23
4H-pyrazolopyrimidin-4-one	10.85	7.03
2-Nonadecanone	24.49	0.66
Gamma-eudesmol	15.22	0.37
Beta-eudesmol	15.66	0.38
Alpha-eudesmol	15.71	0.59
1-Pentanone	22.45	0.47
2-propen-1-one	29.72	15.30
2,3-diphenylcyclopetanone	28.78	0.52
Hydrocarbons and others		
Benzofuran	6.21	0.73
1-buta-1,3-diene	16.81	1.34
3,6 dimethoxy-2-ethylbenzaldehyde	27.35	0.60
4-pyrimidinamine	27.81	0.49
Heptane-2-propanoate	34.68	0.93
3-cyano-5,6-dimethoxy-2-methylthio-1-indole-1-phenylindole	36.57	0.52

RT: Retention Time; TIC: The Ion Current generated depends on the characteristics of the compound concerned and it is not a true quantitation

weight or survival rate ($p>0.05$). Similarly, of the egg quality parameters, neither the Haugh unit nor the egg shell thickness differed significantly between the groups ($p>0.05$).

Information available on the use of propolis in poultry feed shows variations. While some research suggest that propolis has positive effects on performance and egg quality (Bonomi *et al*., 1976; Tatli-Seven, 2008) some other research report the absence of expected effects (Biavatti *et al*., 2003). Nonetheless, Giurgea *et al*. (1981a, b) reported that no clinical sign indicative of any adverse effect of propolis on broiler chickens was observed which may have arisen from the antimicrobial activity of the compounds found in propolis extract. These researchers also suggested that the compounds with antimicrobial effect maintained the health of the digestive tract and improved both digestion and absorption.

In the present study, it was observed that the final live weights measured at the end of the trial of the laying hens given feed supplemented with propolis displayed statistically significant differences. The trial group which was given feed containing propolis at a dose of 400 mg kg^{-1}, displayed the highest live weights. The findings obtained in the present study on body weight gain were in agreement with findings earlier reported by other researchers. In a study conducted by Bonomi *et al*. (1976) in which 18 weeks old laying hens were fed on rations supplemented with 10, 20 and 30 mg kg^{-1} of propolis for a period of 12 months, it was determined that the body weight gain of the group given 30 mg kg^{-1} of propolis improved by 6%. Research conducted in the following years, demonstrated that supplementation of broiler rations with 500 ppm kg^{-1} of propolis increased body weight at a rate of 20% (Marcucci, 1995). Ziaran *et al*. (2005) suggested that as the position of the hydroxyl groups found in the structure of flavonoids displayed similarity to the position of the same compounds in the chemical structure of oestrogens,

Table 3: Effects of dietary propolis on live weight, feed intake, feed efficiency, egg production, egg weight, survival rate, Haugh Unit and shell thickness (n = 60 in each group)

	Propolis				
Parameters	P-0 group	P-1 group	P-2 group	P-3 group	p
Initial body weight (g)	1170.67±1.6500	1171.17±1.5300	1169.83±1.4900	1168.83±1.4300	NS
Final body weight (g)	1717.12±20.960^{ab}	1702.22±22.510^{a}	1726.37±18.670^{ab}	1769.22±19.110^{b}	>0.05
Feed intake (g/day/hen)	109.18±3.8600	108.03±2.9300	110.49±3.9100	111.04±3.8000	NS
Feed efficiency (kg feed/kg egg)	1.932±0.030	1.967±0.023	1.951±0.025	1.943±0.023	NS
Egg production (%)	94.85±0.9600	92.09±0.4300	93.50±0.9700	94.17±0.7600	NS
Egg weight (g)	59.42±2.1400	59.16±1.9900	60.60±2.0700	60.69±2.2000	NS
Survival rate (%)	99.58±0.4200	100.00±0.0000	100.00±0.0000	100.00±0.0000	NS
Haugh unit	69.41±3.0400	68.11±3.3000	70.94±2.4700	69.38±2.8500	NS
Shell thickness (mm)	0.389±0.005	0.387±0.004	0.392±0.005	0.392±0.005	NS

Valuess are expressed as mean±standard deviation; $^{a, b}$Mean values in the same row with different superscripts differ significantly ($p<0.05$); ^{NS}Mean values in the same row that do not differ significantly ($p>0.05$); P-0 group: Control group (basal diet); P-1 group: 100 mg propolis/kg feed; P-2 group: 200 mg propolis/kg feed; 400 mg propolis/kg feed

flavonoids could act similar to anabolic agents with oestrogenic effect and thereby, induce effects similar to those of growth hormones in animals. Furthermore, these researchers suggested that flavonoids could also affect cholesterol synthesis and indicated that the addition of oil extract of propolis to broiler rations at a dose of 100 mg kg^{-1} had positive effect on carcass rates. On the other hand, it has been reported in some other research that propolis supplementation of broiler rations at doses of 40-1000 ppm kg^{-1} (Ziaran *et al*., 2005) and quail rations at doses of 1 and 4 g kg^{-1} (Silici and Guclu-Kocaoglu, 2010) had no effect on live weight gain.

In this study, it was ascertained that when evaluated for all periods, the different doses of propolis added to the ration did not cause any statistically significant difference in the feed consumption ratios of the groups ($p>0.05$). The findings obtained in the present study for feed consumption are contradictory to those obtained in previous research in which propolis was added to laying hen rations at doses of 30 mg kg^{-1} (Bonomi *et al*., 1976), 100 and 150 mg kg^{-1} (Galal *et al*., 2008) and under heat stress conditions 2 and 5 g kg^{-1} (Tatli-Seven, 2008) and to broiler rations at doses of 250 ppm kg^{-1} (Roodsari *et al*., 2004) and 50-250 ppm kg^{-1} (Shalmany and Shivazad, 2006), suggesting that propolis supplementation increased feed consumption rates, attributed to the animals having better health status and feed having better taste owing to the resin, beeswax and vanillin contained in propolis (Bonomi *et al*., 1976). Furthermore, the findings obtained in the present study are in agreement with those obtained in studies in which propolis was added to rations of laying hens at doses of 0.5, 1, 3 and 6 g kg^{-1} (Silici *et al*., 2006), Japanese quails at doses of 1 and 4 g kg^{-1} (Silici and Guclu-Kocaoglu, 2010) and broiler chickens at a dose of 1 mL kg^{-1} (Biavatti *et al*., 2003), suggesting propolis not to have any significant effect on feed consumption.

It was determined that at the end of the trial, the mean feed conversion ratios of the groups for all periods did not display any difference ($p>0.05$). The findings obtained for feed conversion ratios in the present study are in agreement with those obtained with propolis supplementation of broiler (Biavatti *et al*., 2003) and quail (Silici and Guclu-Kocaoglu, 2010) rations, suggesting that propolis does not have any significant effect on feed conversion rates. On the other hand, the results of the present study contradict with those obtained in studies in which propolis was added to rations of laying hens at doses of 30 mg kg^{-1} (Bonomi *et al*., 1976) and under heat stress conditions at 5 g kg^{-1} (Tatli-Seven, 2008), broiler chickens at doses of 200 and 250 mg kg^{-1} (Roodsari *et al*., 2004) and 1000 ppm kg^{-1} (Ziaran *et al*., 2005) and quails at doses of 0.5, 1 and 3 g kg^{-1}, suggesting that propolis improves feed conversion rates.

At the end of the trial, the mean egg production of the groups did not differ from each other significantly ($p>0.05$). This result was in agreement with the report indicating that supplementation of quail rations with 1 and 4 g kg^{-1} of propolis did not have any significant effect on egg production (Silici and Guclu-Kocaoglu, 2010).

Of the egg inner quality parameters, Haugh unit values did not differ significantly between the groups at the end of the trial ($p>0.05$). These findings are in agreement with earlier reports indicating that the addition of propolis to rations of laying hens at doses of 0.5, 1, 3 and 6 g kg^{-1} (Silici *et al*., 2006), quails at doses of 1 and 4 g kg^{-1} (Silici and Guclu-Kocaoglu, 2010), laying hens under heat stress at doses of 2 and 5 g kg^{-1} (Tatli-Seven, 2008) did not affect Haugh unit values. Similarly, the results of the present study demonstrated that the trial groups did not differ from each other for egg shell thickness ($p>0.05$).

It was ascertained that of performance parameters, only live weight gain was affected by supplementation of laying hen rations with propolis. In earlier studies, researchers have attributed live weight gain to the antimicrobial effect of propolis active substances (Glinnik and Gapanovich, 1981) and the flavonoid content of propolis improving digestive system health in return increasing feed consumption (Bonomi *et al*., 1976). In the present study, excluding live weight, performance parameters including feed consumption, feed conversion ratios, egg production and egg weight as well as the overall performance of the groups were not affected by the propolis doses added to the ration. The survival rates of the groups did not significantly differ from each other either. Similarly, of the egg quality parameters, Haugh unit and egg shell thickness values were not affected by the supplementation of laying hen rations with propolis.

CONCLUSION

The propolis doses selected for the supplementation of laying hen rations did not have any significant effect on performance and egg quality parameters, excluding live weight. The researchers consider that further studies are needed for the testing of different propolis doses and their immunological and microbiological effects.

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