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Journal of the Hellenic Veterinary Medical Society

Vol 72, No 2 (2021)

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doi: 10.12681/jhvms.27539

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To cite this article:

ŞENTÜRK, E., YILDIZ, A., OLGUN, O., & MUTLU, M. (2021). Effects of Dietary Supplementation of Propolis on Performance, Egg Quality and Blood Parameters of Layer Quails. *Journal of the Hellenic Veterinary Medical Society*, *72*(2), 2953–2960. https://doi.org/10.12681/jhvms.27539

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J HELLENIC VET MED SOC 2021, 72(2): 2953-2960
ΠΕΚΕ 2021, 72(2): 2953-2960

Research article
Ερευνητικό άρθρο

Effects of Dietary Supplementation of Propolis on Performance, Egg Quality and Blood Parameters of Layer Quails

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ABSTRACT: The aim of this study was to investigate the effect of addition of different levels of propolis to the layer quail diets on performance, egg quality and blood parameters. In the experiment, a total of 120 quails at the age of 22 weeks were randomly distributed to six treatments groups, each with four subgroups. There were 5 quails in each subgroup and the trial lasted 12 weeks. Treatments diets were prepared by adding 0, 2, 4, 6, 8 or 10 g/kg propolis to the basal diet. Body weight change, egg production, egg weight and feed intake were not affected by the addition of propolis to the diet (P>0.05). The egg mass (P<0.01) and feed conversion ratio (P<0.05) linearly improved by addition of propolis. The eggshell breaking strength (P<0.05),eggshell thickness (P<0.01) and Haugh unit (P<0.01) improved by the addition of propolis but other egg quality parameters were not affected (P>0.05).The haematological parameters of the blood were not affected of propolis to the diet (P>0.05), except for the neutrophil content, which was quadratically affected by the addition of propolis, and increased at doses up to 6 g/kg but decreased at the levels of 8 or 10 g/kg (P<0.05). The addition of propolis to the diet did not affect the serum parameters (P<0.05), except cholesterol (P<0.01) and calcium (P<0.05) contents in layer quails. The cholesterol content of serum was linearly affected by the addition of propolis and minimized at the dose of 8 g/kg. The calcium level increased when propolis was supplemented at the dose of 2 g/kg but linearly decreased with higher dose levels. According to these results, it can be said that the addition of 8 g/kg propolis to laying quail diets positively affected the egg mass, eggshell breaking strength, eggshell thickness and cholesterol concentration of serum, however, calcium metabolism was negatively affected by propolis levels.

***Keywords*:** Cholesterol, egg quality, haematological, performance, propolis.

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Date of initial submission: 12-07-2020
Date of revised submission: 15-08-2020
Date of acceptance: 04-09-2020

INTRODUCTION

Prohibition of the use of growth-promoting antibiotics in the poultry industry has started the search for alternative additives to antibiotics. Furthermore, concerns about the possible negative effects of synthetic feed additives in many regions of the world have prompt producers to look for alternative natural feed additives. For these reasons, poultry nutritionists strive to find natural feed additives with beneficial effects on performance, egg quality, meat yield and immune system (Salah Eldin et al., 2015; Raheema, 2016).

It is known that the raw materials, extracts and purified active compounds of bee products commonly used in traditional medicine have antioxidant, antimicrobial and anti-inflammatory properties (Vynograd et al., 2000; De Castro, 2001; Silici and Kutluca, 2005; Aygun et al., 2012; Premratanachai and Chanpen, 2014). Propolis, commonly called bee glue, is a bee product that is collected by worker honey bees (*Apis Mellifera L.*) from tree buds and plant leaks. Honey bees produce propolis by chewing it with enzymes and then mixing it with beeswax and other ingredients (Seven et al., 2010; Banskota et al., 2001; Aygun et al., 2012). Generally, propolis contains resin-polyphenolic fraction (55%), beewax (7%), bee pollen (5%) and aromatic essential oils (30%). In addition to them, it also contains other minor compounds (3%) like vitamins (A, C, D, E, B_1, B_2, B_3, B_6 and B_9) and some minerals such as iron, calcium, copper, nickel, zinc, magnesium, manganese, vanadium, strontium and cobalt (Castaldo and Capasso, 2002; Bankova, 2005). It is rich in vitamins, minerals, enzymes and fatty acids (Lotfy, 2006; Wagh, 2013; Kurek-Górecka et al., 2014). The main ingredients it contains are phenolic compounds, flavonoids and their terpenes and esters (Nolkemper et al., 2010). Besides propolis is used in traditional medicine, it is also used as a dietary supplement in poultry diets. There are research results indicating that propolis when added into broiler and laying hens diets can enhance the performance (Shalmany and Shivazad, 2006; Seven et al., 2010; Seven et al., 2012), improve some serum parameters (Galal et al., 2008; Attia et al., 2014) and decrease oxidative stress (Mannaa et al., 2011; Hosseini et al., 2015). According to Galal et al. (2008) propolis use in laying hens nutrition has positive effects on performance and egg quality, and these effects are due to its high palatability. It also contains compounds that increase the digestion and absorption of calcium, and it was stated to improve eggshell quality (Seven, 2008; Seven et al., 2011). It is also reported that it prevents anemia by increasing red blood cells in poultry (Khan, 2017). Considering the aforementioned beneficial effects of propolis in broilers and laying hens we hypothesized that similar effects could also be seen in quails

This research was carried out to investigate the effect of supplementing diets with different levels of propolis on performance, egg quality, and the haematological or biochemical properties of blood in Japanese quails.

MATERIALS AND METHODS

Ethical Approval

The animal care practices were used in the experiment in consistency with animal welfare rules stated in Article 9 in government law in Turkey (No. 5996)

Animals and Diets

This study was carried out with a total of 120 female Japanese quails at the age of 22 weeks and lasted 12 weeks. Quails were randomly distributed to six experimental groups. Each trial group was divided into four subgroups, each containing five quails. Treatment diets were prepared with six increasing levels of propolis (0, 2, 4, 6, 8 and 10 g/kg). Chemical analysis values of propolis used in the research are as follows: 39.95% mechanical mass, 2.54% beewax, 46.63% soluble solids, 3.31% flavonoid expressed in quercetin 3.75% minerals, 7.57% humidity. Propolis supplied from Konya, Turkey were cut into small pieces and frozen at -20 ^{o}C. Then, the frozen propolis was pulverized in the blender by adding double its weight of corn starch. The basal diet was prepared according to NRC (1994). The basal diet and its calculated nutrient content are shown in Table 1. Throughout the experiment, feed and water were given to quails *ad-libitum*.

Determination of Performance Parameters

Body weight change was determined by group weighing at the beginning and end of the experiment. Feed intake was calculated as g/d/quail. Egg production of birds was recorded daily and calculated as %. Egg weight was obtained by weighing the eggs collected in the last three days of the experiment. Egg mass was calculated from formula: *(egg production (%) x egg weight)/100*.The feed conversion ratio was calculated using the following formula: *feed intake (g feed/quail)/egg mass (g/egg/quail)*.

Table 1. Basal diet and its calculated nutrient content

Ingredients	g/kg	Nutrient contents	g/kg
Corn	542	Metabolisable energy, kcal ME/kg	2902
Soybean meal	270	Crude protein	200.9
Sunflower meal	70	Calcium	25.1
Sunflower oil	43	Available phosphorus	3.5
Limestone	56	Lysine	10.0
Dicalcium phosphate	11.5	Methionine	4.5
Salt	3.5	Cystine	3.7
Premix[1]	2.5	Methionine+cystine	8.2
DL methionine	1.5		
Total	1000.0		

[1]Premix is supplied that per kg of diet; Manganese: 80 mg, Iron: 60 mg, Copper: 5 mg, Iodine: 1 mg, Selenium: 0.15 mg, Vitamin A: 8.800 IU, Vitamin D_3: 2.200 IU, Vitamin E: 11 mg, Nicotine acid: 44 mg, Cal-D-Pan: 8.8 mg, Riboflavin: 4.4 mg, Thiamine: 2.5 mg, Vitamin B_{12}: 6.6 mg, Folic acid: 1 mg, Biotin: 0.11 mg, Choline: 220 mg.

Determination of Egg Quality Parameters

The egg quality parameters were determined by analysis of eggs collected in the last three days of the experiment. Egg length and egg diameter of each egg was measured by digital calliper. Egg shape index was calculated with (egg diameter / egg length) x 100 formula. Eggshell breaking strength was measured by applying supported systematic pressure to blunt of the eggs (Egg Force Reader, OrkaFood Technology, Israel). Afterwards, the yolk diameter was measured with a digital calliper and the yolk height was measured with a digital height gauge. The yolk index was obtained by using these parameters with *(yolk height / yolk diameter) x 100* formula. Similarly, length and diameter of albumen were determined with digital calliper and albumen height was determined with digital height gauge. Using these parameters, the albumen index was found from [*albumen height / (albumen length + albumen diameter) / 2)] x 100*. Additionally, Haugh unit was calculated as follows: Haugh unit = *100 x log (albumin height + 7.57-1.7 x egg weight*$^{0.37}$) (Haugh, 1937). The egg quality analyses were completed within 24 hours after eggs were collected. The ratio of eggshell weight including the membrane to egg weight was determined by using *eggshell weight (g) /egg weight (g) x 100* formula. The thickness of eggshell including the membrane was calculated from the values obtained with digital calliper from three sections of the eggs.

Determination of Haematological and Biochemical Parameters of Blood

At the end of the study, two quails from each subgroup (eight quails per treatment group) were randomly selected and blood samples (2 ml per quail) were taken either into heparinised tubes for haematological analyses or in centrifuge tubes for biochemical constituents analyses. The analyses of blood and serum samples were made by an auto-analyser.

Statistical Analysis

Data were analysed in the SPSS 18.0 software package (SPSS Inc., Chicago, IL, USA) with model of one-way ANOVA, using the group mean as an experimental unit. A probability value of $P<0.05$ was considered statistically significant. Orthogonal polynomial contrasts were used to evaluate the significance of linear and quadratic models to determine the response of the dependent variable to an increasing propolis level.

RESULTS

Performance

The body weight change, egg production, egg weight, egg mass, feed intake and feed conversion ratio are shown in Table 2. The supplementation of different levels of propolis to diets did not significantly affect body weight change, egg production, egg weight and feed intake ($P>0.05$). Egg mass increased linearly ($P<0.01$) when propolis was added to quail diets. Feed conversion ratio was affected linearly ($P<0.05$) with the supplementation of increasing levels propolis to diets in quails.

Egg Quality Parameters

The damaged eggs, egg shape index, eggshell breaking strength, eggshell weight and thickness or albumen and yolk indexes and Haugh unit results are presented in the Table 3.Some of the egg internal and external parameters such as damaged eggs, egg shape index, eggshell weight and yolk index were not significantly affected by treatment diets (P>0.05). The addition of propolis to the diets linearly increased the eggshell breaking strength which was maximized at the dose level of 8 g/kg (P<0.05). Similarly, eggshell thickness was considerably enhanced by increasing levels of propolis, especially at the level of 8 g/kg (P<0.01). The albumen index decreased by supplementation with propolis and was minimized at the dose of 6 g/kg (P<0.05). Haugh unit also decreased at the dose of 6 g/kg level, but it was surprisingly maximized at the level of 10 g/kg (P<0.05).

Table 2. Effect of dietary supplementation of propolis on the performance parameters in layer quails

Parameters	Propolis (g/kg)							*P*- value of contrast	
	0	2	4	6	8	10	SEM*	Linear	Quadratic
Body weight change, g	26.42	19.50	21.58	18.33	24.58	12.25	4.906	0.203	0.833
Egg production, %	86.94	92.59	90.59	91.14	91.89	93.83	1.893	0.062	0.681
Egg weight, g	12.36	12.40	12.44	12.16	12.76	13.37	0.327	0.061	0.130
Egg mass, g/d/quail	10.75	11.48	11.28	11.08	11.70	12.56	0.343	0.010	0.292
Feed intake, g/d/quail	32.84	33.61	34.15	32.71	33.32	33.86	1.025	0.752	0.928
Feed conversion ratio, g feed/g egg	3.06	2.93	3.05	2.95	2.85	2.71	0.096	0.020	0.304

*Standard error of mean

Table 3. Effect of dietary supplementation propolis on the egg quality in layer quails

Parameters	Propolis (g/kg)							P value of contrast	
	0	2	4	6	8	10	SEM*	Linear	Quadratic
Damaged eggs, %	0.58	0.15	0.43	0.15	2.08	0.74	0.619	0.407	0.802
Egg shape index	76.37	79.62	77.30	78.72	77.22	77.92	1.253	0.878	0.503
Eggshell breaking strength, kg	1.29	1.44	1.42	1.33	1.51	1.47	0.045	0.021	0.763
Eggshell weight, % of egg weight	7.22	7.66	8.03	7.44	7.65	7.84	0.168	0.121	0.289
Eggshell thickness, µm	169	186	201	197	220	212	4.3	<0.001	0.045
Albumen index	6.04	5.09	5.52	4.89	5.46	5.93	0.284	0.984	0.018
Yolk index	52.16	51.08	51.38	47.92	50.81	50.71	1.427	0.357	0.269
Haugh unit	71.69	67.44	69.56	65.07	70.03	73.53	2.152	0.502	0.030

*Standard error of mean

Haematological Parameters of Blood

The haematological parameters of blood are demonstrated in Table 4. In the current study, white blood cell, lymphocyte, red blood cell, haemoglobin, haematocrit, erythrocyte volume, mean corpuscular haemoglobin, corpuscular haemoglobin concentration, red blood cell distribution width and haemoglobin/lymphocyte ratio were not affected by experimental diets (P>0.05). Among the haematological parameters, only the neutrophil concentration was quadratically affected by supplementation of quail diets with propolis, reaching a maximum at the level of 6 g/kg (P<0.05). However, it was diminished significantly by further supplementation of propolis.

Biochemical Analysis of Serum

The serum biochemical parameters are shown in Table 5. No treatment effect was observed except for the cholesterol and calcium concentrations of (P>0.05). The cholesterol and calcium levels were linearly affected (P<0.01 and P<0.05, respectively) by the addition of propolis to diets. The cholesterol concentration was decreased by dietary addition of propolis while its lowest concentration was obtained from the group of quails that received 8 g/kg. Besides, the calcium level reached a maximum at the dose of 2 g/kg while it decreased at higher levels.

Table 4. Effect of dietary supplementation with propolis on the haematological parameters in layer quails

Parameters	Propolis (g/kg)						SEM*	*P*- value of contrast	
	0	2	4	6	8	10		Linear	Quadratic
WBC, 10^3/µl	9.04	14.65	13.87	13.40	14.15	11.94	2.108	0.518	0.129
NEU, 10^3/µl	0.65	0.75	1.13	2.18	0.33	0.61	0.237	0.858	0.050
LYM, 10^3/µl	9.08	13.33	11.76	7.70	11.27	8.65	1.691	0.411	0.403
RBC, 10^6/µl	3.13	4.31	3.25	3.00	3.24	3.18	0.205	0.142	0.655
HGB, g/dL	17.60	19.97	17.67	16.30	18.00	17.15	0.687	0.140	0.986
HCT, %	46.20	55.10	45.65	42.13	44.25	44.70	2.230	0.061	0.868
MCV, µm^3	148	146	141	141	142	141	2.7	0.088	0.341
MCH, pg	56.33	53.77	54.35	54.57	56.20	53.92	1.565	0.761	0.729
MCHC, g/dL	38.13	36.50	38.70	38.90	40.77	38.37	0.846	0.081	0.550
RDW, %	10.33	10.72	9.62	10.63	11.25	10.22	0.449	0.707	0.971
HGB/LYM	2.10	1.65	1.73	2.86	1.66	2.07	0.425	0.808	0.852

WBC: White Blood Cell, NEU: Neutrophil, LYM: Lymphocyte, RBC: Red Blood Cell, HGB: Haemoglobin, HCT: Haematocrit, MCV: Erythrocyte Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Corpuscular Haemoglobin Concentration, RDW: Red Blood Cell Distribution Width, PLT: Thrombocyte, HGB/LYM: Haemoglobin/Lymphocyte Ratio.
*Standard error of mean

Table 5. Effect of dietary supplementation with propolis on the biochemical parameters of serum in layer quails

Parameters	Propolis (g/kg)						SEM*	*P*- value of contrast	
	0	2	4	6	8	10		Linear	Quadratic
Total protein, g/dL	4.47	5.42	4.17	4.35	4.30	4.00	0.310	0.052	0.627
Albumin, g/dL	1.50	1.75	1.42	1.45	1.55	1.32	0.098	0.110	0.484
Globulin, g/dL	2.97	3.67	2.62	2.87	3.22	2.65	0.219	0.168	0.714
Creatinine, mg/dL	0.35	0.36	0.37	0.34	0.34	0.35	0.010	0.657	0.738
Glucose, mg/dL	319	344	332	335	333	357	13.05	0.167	0.752
Cholesterol, mg/dL	250	212	183	166	138	149	16.9	<0.001	0.139
ALT, U/L	3.25	2.75	2.50	2.75	2.50	3.00	0.387	0.618	0.203
AST, U/L	219	219	220	242	258	240	17.16	0.118	0.866
Ca, g/dL	26.82	30.46	24.07	25.60	26.42	22.25	1.560	0.034	0.531
P, g/dL	8.02	8.52	6.22	7.40	7.52	6.80	0.896	0.319	0.679

ALT: Alanine aminotransferase AST: Aspartate aminotransferase.
*Standard error of mean

DISCUSSION

Performance

The results obtained from this study are in agreement with published studies claiming that the supplementation of different levels of propolis to the diets of birds positively influenced egg mass (Galal et al., 2008; Tatli Seven, 2008; Belloni et al., 2015; Mehaisen et al., 2019). The egg mass was enhanced by increasing dietary propolis and the highest value was observed at 10 g/kg level of propolis. Also, Mehaisen et al. (2019) stated that the egg mass increased by addition of 1 g/kg propolis in quails. Similar results have been reported by Belloni et al. (2015) and Soltani et al. (2019).In the present study, feed conversion ratio of laying quails improved by the supplementation of propolis in the diet, especially at the dose levels of 8 and 10 g/kg. This result agree with Zeweil et al. (2016) and Mehaisen et al. (2019), who showed that the addition of propolis (250-1000 mg/kg) to the diet improved feed conversion ratio in laying quails. At the same time, Galal et al. (2008) demonstrated that the feed conversion ratio of hens improved by dietary addition of propolis (100-150 mg/kg). Similarly, Tatli Seven (2008) reported that the supplementation of 2 or 5 g/kg propolis to the diet enhanced the feed conversion ratio in laying hens. In parallel, Belloni et al. (2015) noted that the feed conversion ratio of laying hens improved by the supplementation of 3% propolis. The positive effect of propolis on performance parameters such as egg mass and feed conversion ratio may be due to its potential to increase the digestibility and absorption of nutrients by promoting certain biological activities (Marieke et al. 2005; Kročko et al.2012). ShreifEffat and El-Saadany (2016) also reported that the high flavonoids and phenolic acids contained in propolis may increase the absorption of crude protein and other nutrients by improving the gut microflora. Additionally, it may be propolis content of flavonoids that exerts antimicrobial and immunomodulatory effects and reduces the level of detrimental oestrogen (Burdock, 1998; Shaddel-Tili et al., 2017; Duarte et al., 1993; Hanasaki et al., 1994; Middleton and Kandaswami, 1994).

Egg Quality

In current study, the eggshell breaking strength and eggshell thickness increased by supplementation of propolis to the diets. The present results agree with those of previous studies (Galal et al., 2008; Tatli Seven et al., 2008; ShreifEffat and El-Saadany, 2016; Mehaisen et al., 2019; Soltani et al., 2019). Mehaisen et al. (2019) noted that the eggshell thickness increased by dietary addition of 1 g/kg level propolis in quails. Galal et al. (2008) reported that the eggshell thickness of laying hens was increased by supplementation of 100-150 mg/kg propolis to diets. For all that, Tatli Seven (2008) showed that the 2 or 5 g/kg of propolis positively affected the eggshell thickness in hens. Similar results have been reported by ShreifEffat and El-Saadany (2016) and Soltani et al. (2019). The enhanced eggshell thickness and eggshell breaking strength may be associated with the increased digestibility and absorption of calcium and phosphorus as a result of acid derivatives such as benzoic, 4-hydroxy-benzoic, etc., which are found in propolis (Haro et al., 2000). The albumen index diminished with the propolis supplementation to diets, especially 6 g/kg level. These results disagree with previous reports that the addition of propolis to the diet does not affect the albumin index (Tatli Seven, 2008; ShreifEffat and El-Saadany, 2016; Zeweil et al., 2016; Mehaisen et al., 2019). The Haugh unit is accepted as an index to predict the egg quality (Monira et al., 2003). At the present study, Haugh unit quadratically diminished with the addition of propolis to the diets, except for the 8 and 10 g/kg level. The highest Haugh unit was observed at the 10 g/kg level of propolis. Similarly, ShreifEffat and El-Saadany (2016) stated that dietary addition of 150, 300 or 450 mg/kg levels of propolis to the diet improved Haugh unit in laying hens. Additionally, Galal et al. (2008) and Abdel-Kareem and El-Sheikh (2017) reported that the Haugh unit was positively affected by the addition of propolis to the diet.

Blood Parameters

The propolis treatment did not affect haematological parameters linearly or quadratically, except for neutrophil concentration that quadratically increased for doses up to 6 g/kg of diet. Neutrophils represent the vast majority of white blood cells that consist part of the warrior cells of the defence system that protects the organism from various microbes and harmful toxins. Although there are some results about white blood cells and their sub-types such as lymphocyte, monocyte, eosinophil and basophil, among the studies carried out, there is no finding related to neutrophil count (Abdel-Rahman and Mosaad, 2013; Omar et al., 2003; Shaddel-Tili et al., 2017; Hassan et al., 2018). In the present study, cholesterol and calcium concentrations in serum were significantly affected by supplementation of propolis. The cholesterol level was linearly decreased and this result is in agreement with previous reports by Galal et al. (2008), Zeweil et al. (2016) and Abdel-Kareem and El-Sheikh (2017) who tested dietary inclusion of propolis at doses ranging from 100 to 1000 mg/kg of diet. However, there were also results indicating that serum cholesterol concentration was not affected by the addition of propolis to the diet (Denli et al., 2005; Soltani et al., 2019). Tatli Seven et al. (2008) and Arpasova et al. (2016) noted that these different results may be partially originated from variations in compositions of propolis as a result of collection area and time and bee flora. The calcium concentration was the highest at the dose of 2 g/kg and it decreased at higher levels of propolis. Contrary to the current study, Mehaisen et al. (2019) reported that the serum calcium level increased with the addition of propolis. Additionally, the calcium concentration of serum significantly decreased with the supplementation of propolis to quail diets and the lowest concentration was observed at 10 g/kg propolis level. The reason for the decrease in the serum concentration of calcium, which is the main component of the eggshell, may be that propolis uses calcium more effectively for the purpose of improving eggshell resistance and thickness.

CONCLUSION

In conclusion, it can be said that propolis, which is a natural feed additive, positively affected performance, eggshell quality, and cholesterol concentration of serum in layer quails but negatively affected the Ca metabolism at levels equal or higher than 4 g/kg.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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