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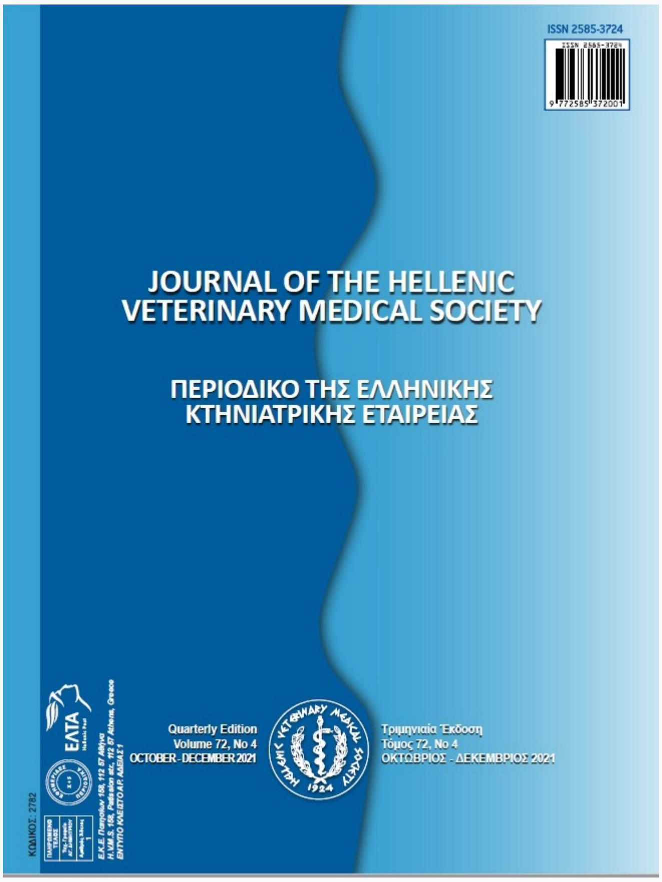

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Research article
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Effect of the addition of bee pollen to the diet on performance, eggshell quality and serum parameters in layer quails

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ABSTRACT: This study was carried out to determine the effect of bee pollen addition to quail diets on performance, eggshell quality and serum biochemical properties. In the experiment, 120 quails aged 70 days were randomly distributed to 6 treatment groups with 4 replicates. The quails were fed for 10 weeks with 6 trial diets with 0, 2, 4, 6, 8 and 10 g/kg bee pollen added. The body weight change, egg production, egg weight, egg mass, feed conversion ratio, damaged eggs, eggshell weight, eggshell thickness and serum glucose, creatinine, albumin, globulin and total protein levels of quails were not affected by the addition of bee pollen to the diet (P>0.05). The addition of 10 g/kg bee pollen to the diet significantly decreased feed intake compared to the control group (0 g/kg). Eggshell breaking strength was decreased by adding bee pollen to the diet and the lowest value was observed at the group fed the diet containing 6 g/kg bee pollen.Serum cholesterol concentration was minimized when 10 g/kg bee pollen was added to the diet(P<0.05). In addition, serum calcium and phosphorus contents were increased compared to the control (0 g/kg) group and these parameters reached the maximum at the 10 g/kg bee pollen level (P<0.01).According to the results of the experiment, the addition of bee pollen to the diet had a positive effect on serum cholesterol, calcium and phosphorus levels in layer quails, however, it had a negative effect on eggshell breaking strength.

***Keywords*:** Bee pollen, quail, performance, eggshell quality, cholesterol.

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INTRODUCTION

In recent years, the use of alternative additives to antibiotics has increased to improve the immunity and performance of animals. One of the candidate products for these additives is bee pollen (Farag and El-Rayes, 2016).

Bee pollen is a bee product formed by mixing flower pollen collected by worker bees with digestive enzymes such as beta-glycosidase (Carpes et al., 2008). Bee pollen is rich in carbohydrates (13-55%), protein (10-40%), fatty acids (1-20%), vitamins (0.02-0.10%) and minerals (0.5-3%) as well as flavonoids (0.04-3%) (Villanueva et al.,2002;Carpes et al., 2007; Isidorov et al.,2009; Taha, 2015).However, the nutrients and active substances contained in bee pollen vary according to available flowers or, in other words, to the flora around the hive (Taha, 2015).

Bee pollen is reported to have anti-microbial, anti-inflammatory (Kacaniova et al.,2012;Pascoal et al., 2014), anti-fungal (Garcia et al.,2001), anti-allergic (Moita et al., 2014), anti-viral, hypolipidemic, hypoglycaemic and immune-enhancing effects (Komosinska-Vassev et al., 2015).Desoky and Kamel (2018) stated that the addition of bee pollen (2 g/kg) to the diet increased feed intake (FI), egg mass (EM), eggshell thickness and serum total protein, albumin and globulin levels, and decreased feed conversion rate and serum cholesterol level in laying quails. Rizk et al. (2018) reported that the feed efficiency of laying hens was maximized by adding 1000 mg/kg of bee pollen to their diets, however, the addition of 500 mg/kg to diet was more economically appropriate.Similarly, in another study, it was reported that with the supplementation of bee pollen to the diet, feed efficiency in laying hens enhanced and as a result, producer profit increased (Demir and Kaya, 2020). However, it has been reported that supplementation of bee pollen (2.5, 5.0 or 7.5 g/kg) to the diet does not affect performance in laying quails (Al-Hamdani and Al-Douri, 2018). In addition, some research results showed that the addition of bee pollen to the diet was effective in changing blood biochemistry parameters, especially lowering serum cholesterol level (Fazayeli-Rad et al.,2015; Farag and El-Rayes, 2016; Rizk et al., 2018; Demir and Kaya, 2020).

Research results show that addition of bee pollen to the diet has a positive effect on poultry, but the number of studies on the effect of addition of bee pollen to the diet in layer birds is very few, and the optimum level of bee pollen in the diet is still uncertain. Therefore, it is necessary to increase the number of studies.

This research was carried out to determine the effect of bee pollen additionto layer quail diets on production performance, eggshell quality and serum biochemical parameters.

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 Feed Materials

In the experiment which lasted 70 days, 120 female Japanese quails at the age of 10 weeks were used. The quails were assigned to six treatment groups with four replicates, each with five quails. The six treatment diets used in the experiment were prepared by adding bee pollen at the 0, 2, 4, 6, 8 or 10 g/kg levels to the control diet (Table 1) which was formulated according to NRC (1994) nutrient requirements for layer quails. The bee pollen used in the experiment was collected from Konya region, and it contains 14.3% moisture, 2.1% crude ash, 19.4% crude protein, 7.4% crude fat, 13.2% fiber and 56.9%carbohydrate (Başdoğan et al., 2019).During the experiment, feed and water were given to quails *ad-libitum*and a lighting program of 16 hours/day was applied.

Determination of performance parameters

Body weights of quails were determined by group weighing at the beginning and the end of the experiment, and body weight change (BWC) was determined from these values. At the end of the experiment, feed intake (FI) was calculated as g/day/quail. Egg production (EP) was recorded daily and the percentage of egg production per quail was calculated for the total experimental period.Egg weight (EW) was obtained by weighing all eggs collected in the last three days of the research. Egg mass was determined using this formula: EM = EP (%) x EW) / 100.The feed conversion ratio was calculated by dividing FI (g/d/quail) by the EM (g/egg/quail).

Determination of eggshell quality parameters

All eggs collected in the last three days (on days 68-70) of the experiment were used to determine the eggshell quality. The collected eggs were firstly weighed, and then the eggshell breaking strength (kg)

Table 1. Basal diet and its calculated nutrient contents

Ingredients	g/kg	Nutrient contents	g/kg
Corn	542.0	Metabolisable energy, kcal ME/kg	2902
Soybean meal (47%)	270.0	Crude protein	200.9
Sunflower meal (36%)	70.0	Calcium	25.1
Sunflower oil	43.0	Available phosphorus	3.5
Limestone	56.0	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 provide 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 D3: 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 B12: 6.6 mg, Folic acid: 1 mg, Biotin: 0.11 mg, Choline: 220 mg.

Table 2. Effects of bee pollen addition to diet on performance parameters in layer quails

Parameters	Bee Pollen, g/kg						SEM*	*P value*
	0	2	4	6	8	10		
BWC, g	14.33	26.08	17.08	18.54	20.83	13.00	5.728	0.762
EP, %	81.28	88.01	88.49	86.98	82.18	80.66	2.348	0.096
EW, g	13.09	12.89	12.64	12.54	12.58	12.86	0.344	0.895
EM, g/egg/quail	10.64	11.34	11.16	10.91	10.34	10.38	0.390	0.441
FI, g/d/quail	32.51[a]	32.76[a]	33.18[a]	32.65[a]	31.17[ab]	30.47[b]	0.610	0.050
FCR, g feed/g egg	3.06	2.90	2.98	3.00	3.03	2.96	0.105	0.936

BWC: Body weight change, EP: Egg production, EW: Egg weight, EM: Egg mass, FI: Feed intake, FCR: Feed conversion ratio.
*Standard error means.
[ab]Values bearing different superscript in rows are statistically significant (P<0.05).

was measured by applying supported systematic pressure (Egg Force Reader, OrkaFoodTechnology, Israel) to the blunt of the eggs. Afterwards, the eggs were emptied and the eggshells were washed so that no egg whites remained in tap water and dried at room temperature (24 ^{o}C) for three days. The dried eggshells were weighed by a precision scale with 0.01 g delicate, divided into the egg weight, and the eggshell rate was calculated as% of the egg weight.Membrane eggshell thickness (µm) was determined with a micrometer by taking three measurements from blunt, middle and pointed sections of eggshells.

Determination of serum parameters

At the end of the experiment, one quail from each subgroup (24 quails in total) was randomly selected and used for blood sampling by the method of heart puncture.Blood samples were centrifuged at 3000 rpm for 5 minutes to obtain serum samples that were stored at -20 ^{o}C until analysed. The evaluation of serum biochemical constituents was made by an auto-analyser (Beckman LX-20 Coulter, Ireland) and using commercial kits (Beckman, Ireland).

Statistical analysis

The variance analyses have been applied to all variables obtained from the trial groups (Minitab, 2000), and the differences between means of the groups were determined by the Duncan (Duncan, 1955) multiple comparison test.

RESULTS AND DISCUSSION

Performance Parameters

The effects of bee pollen addition to the diets on performance parameters in layer quails are given at Table 2. The supplementation of bee pollen to quail diets did not statistically affect BWC, EP, EW, EM and FCR (P> 0.05).

Feed intake was significantly affected by bee pollen supplementation to the diet, and the FI was significantly lower in the group that received bee pollen at a high level (10 g/kg) than in the groups supplemented with 0 (control group), 2, 4 and 6 g/kg bee pollen (P = 0.05). Similarly, Demir and Kaya (2020) reported that the addition of adding 0.5, 1.0 or 1.5% bee pollen to laying hen diets caused a linear decrease in FI.

In a study conducted with broilers, it was stated that FI declined by adding 0.2, 0.4 or 0.6% bee pollen to the diets (Farag and El-Rayes, 2016). Hosseini et al. (2016) also reported that the supplementation of bee pollen (20 g/kg) to broiler diets reduced FI.Rizk et al. (2018) demonstrated that there was no difference between the groups in terms of FI by adding bee pollen at levels of 0, 500, 1000 or 1500 mg/kg to laying hen diets. Similar results were reported in another study in which 0.5% palm pollen was used in diets (Mousa et al., 2018). However, Desoky and Kamel (2018) showed that the addition of 2 g/kg level of bee pollen to the diet increased FI in laying quails. Similar results were reported by Canoğulları et al. (2009) in growing quails. It is seen that some studies are in agreement with the results of current research in terms of FI (Farag and El-Rayes, 2016; Hosseini et al., 2016; Demir and Kaya, 2020), but some studies results in the literature are not compatible with present research (Canoğulları et al., 2009; Mousa et al., 2018;Rizk et al., 2018). The decrease in feed intake when high levels of pollen (10 g/kg) are added to the diet may be due to the low palatability of the pollen.

Eggshell Quality Parameters

The effects of adding different levels of bee pollen to the diets on eggshell quality parameters in layer quails are demonstrated at Table 3.

The treatments did not statistically affect the eggshell quality parameters (P>0.05), except for eggshell breaking strength (P<0.05). The highest eggshell breaking strength was obtained in the control (0 g/kg) group, and only a difference between this group and the group that was supplemented with 6 g/kg bee pollenwas observed.Demir and Kaya (2020) stated that the addition of 1.5% bee pollen to laying hen diets did not affect eggshell parameters, including eggshell breaking strength.Similar results were also reported by Rizk et al. (2018).Also, Desoky and Kamel (2018) noted that addition of 2 g/kg level bee pollen to the diet did not affect the eggshell weight of the eggshell quality parameters, but increased the eggshell thickness in laying quails. These results are not in agreement with the current study results. Differences in animal material used and diet pollen level can be listed as possible causes of incompatibility.

Serum Parameters

The effects of adding different levels of bee pollen to the diets on serum biochemical constituents in layer quails are demonstrated at Table 4. The effect of bee pollen addition to the quail diets was statistically insignificant on serum glucose, creatinine, albumin, globulin and total protein (P> 0.05).

Table 3. Effects of bee pollen addition to diet on egg quality parameters in layer quails

Parameters	Bee Pollen, g/kg						SEM*	*P value*
	0	2	4	6	8	10		
Damaged eggs, %	1.68	0.00	0.97	1.21	1.71	2.44	0.996	0.768
Eggshell breaking strength, kg	1.52^{a}	1.46^{ab}	1.38^{ab}	1.24^{b}	1.37^{ab}	1.33^{ab}	0.048	0.022
Eggshell weight, % of EW	7.91	7.64	7.70	7.55	7.79	7.96	0.197	0.777
Eggshell thickness, µm	198	193	196	197	202	201	3.803	0.594

* Standard error means.
^{ab}Values bearing different superscript in rows are statistically significant (P<0.05).

Table 4. Effects of bee pollen addition to diet on serum biochemical constituents in layer quails

Parameters	Bee Pollen, g/kg						SEM*	*P value*
	0	2	4	6	8	10		
Glucose, mg/dL	274	286	305	282	276	280	6.4	0.066
Cholesterol, mg/dL	213^{ab}	239^{a}	196^{ab}	221^{ab}	179^{ab}	153^{b}	13.6	0.019
Total protein, g/dL	4.27	4.37	4.35	4.27	4.20	4.70	0.261	0.829
Creatinine, mg/dL	0.33	0.34	0.34	0.32	0.32	0.32	0.010	0.624
Albumin, g/dL	1.43	1.47	1.37	1.42	1.37	1.60	0.062	0.172
Globulin, g/dL	2.83	2.90	3.07	2.85	2.65	3.10	0.186	0.696
Calcium, mg/dL	22.50^{B}	25.17^{AB}	26.32^{A}	23.00^{AB}	23.80^{AB}	25.95^{A}	0.731	0.007
Phosphorus, mg/dL	5.47^{b}	5.87^{b}	5.83^{b}	5.48^{b}	6.87^{ab}	8.30^{a}	0.592	0.040

* Standard error means.
^{AB}Values bearing different superscript in rows are statistically significant (P<0.01).
^{ab}Values bearing different superscript in rows are statistically significant (P<0.05).

Serum cholesterol reduced as the level of bee pollen added to the diets increased and it was minimal in the group fed diets containing 10 g/kg bee pollen. In terms of serum cholesterol levels, the difference between the groups supplemented with 2 g/kg and 10 g/kg bee pollen was found statistically significant (P <0.05), while the serum cholesterol levels of the other groups were similar to these two groups. Desoky and Kamel (2018) indicated that the supplementation of bee pollen at the level 2 g/kg to the diet decreased serum cholesterol level of laying quails. Similarly, Demir and Kaya (2020) reported that serum cholesterol decreased linearly in laying hens by adding bee pollen (0, 0.5, 1.0 or 1.5%) to the diet. Similar results were stated in the results of researches conducted with broilers (Fazayeli-Rad et al., 2015; Farag and El-Rayes, 2016), roosters (Abuoghaba and Ismail, 2018) and laying hens (Rizk et al., 2018). In the present study, although there was no difference with the control group, there was a high decrease in serum cholesterol level with the addition of bee pollen at levels of 8 and 10 g/kg to the diet, 15.96% and 28.17%, respectively.Rizk et al. (2018) reported that there was a decrease in serum cholesterol levels with the addition of bee pollen to the diets due to the high levels of unsaturated fatty acids such as oleic, linoleic and linolenic acids contained in bee pollen.

The effect of diet supplementation with bee pollen on serum calcium level was statistically significant (P<0.01). The serum calcium levels of quails that received 4 and 10 g/kg bee pollen were the highest while the respective level in quails of the control group was the lower. The effect of bee pollen supplementation on serum phosphorus levels in the diets of laying quails was statistically significant (P<0.05). The highest serum phosphorus level was detected in the group supplemented with 10 g/kg bee pollen and was statistically significant compared to values of groups supplemented with 0, 2, 4 and 6 g/kg bee pollen.In previous studies, it was stated that the serum calciumlevel was not affected by the addition of pollen to the diet (Desoky and Kamel, 2018; Demir and Kaya, 2020), and the serum phosphorus level was not affected (Desoky and Kamel, 2018) or decreased (Demir and Kaya, 2020) in layer birds. These results are in disagreement with the results of current research. Farag and El-Rayes (2016) stated that the bee pollen contains 0.35% calcium and 0.03% phosphorus. It can be said that this increase in serum calcium and phosphorus levels may have been caused by the calcium and phosphorus contained in bee pollen. However, it should be noted that the increase in serum mineral level does not reflect positively on the eggshell quality.

CONCLUSION

The addition of bee pollen to laying quail diets negatively affected feed intake (10 g/kg) and eggshell breaking strength (6 g/kg), and positively affected serum calcium and phosphorus levels. Since bee pollen tends to decrease the serum cholesterol level in quails, it can be said that it is beneficial to conduct further researches on the pollen-cholesterol relationship.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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