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Effects of supplemental bee pollen on performance, meat quality, serum constituents and immunity system in growing quails

Behlül Sevim
Department of Veterinary, Eskil Vocational School, Aksaray University, Aksaray, 68800, Turkey

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Abstract

This study focused on the effects of adding various levels of bee pollen to diets for Japanese quail *(Coturnix coturnix Japonica)*. The response variables that were examined included their performance, carcass characteristics, meat colour, immune system, and some serum constituents. A total of 160 one-day-old quail chicks were used. These chicks were randomly assigned to four treatments, each consisting of 40 chicks. These groups were further divided into four replicates of ten birds. The treatments consisted of diets to which 0.0, 2.5, 5.0, and 10 g/kg bee pollen were added. The feeding period lasted 42 days. The performance and serum biochemistry of the growing quails were not affected by the addition of bee pollen to the diet. The addition of 5 g/kg bee pollen increased the follicle weight and the L^* value of the breast, and decreased the a^* value of the thigh. It also improved the serum immunoglobulin A (IgA) content. Thus, the addition of 5 g/kg bee pollen to the diet of growing quails was effective in improving follicle development, meat quality and immune system.

Keywords: bee pollen, carcass, immunity, meat quality, quail, performance, serum
Corresponding author: behluls68@gmail.com

Introduction

Pollen is the male reproductive part of flowering plants. After it has been collected and dried by honeybees it is referred to as 'bee pollen' (Erdoğan & Dodoloğlu, 2005). The chemical composition of bee pollen varies depending on the geographical region and plant diversity. It generally contains 25 - 30% protein, 30 - 55% carbohydrates, 1 - 20% fatty acids, and lipids, such as sterols, phenolic acids, flavonoids, vitamins, and minerals (Silici, 2014; Martiniakova *et al.*, 2021). The bioactive substances of bee pollen are responsible for its anti-inflammatory, antioxidant, immune-stimulating, and immune-modulatory effects in animals (Prakatur *et al*., 2020).

Intensive poultry breeding has provided for more live weight per unit of feed consumption. However, there is a negative relationship between performance and disease resistance in poultry, and as performance increases, their immune system can be suppressed. Additionally, factors such as the nutritional content of the diet affect the formation of antibodies and the development of the immune system (Sarıca *et al*., 2009).

In recent years, the importance of feed additives in preventing and treating disease has increased. Natural feed additives such as bee products, probiotics and plant extracts are used more frequently in poultry production. These substances may provide physiological benefits to the body and reduce the risks of disease. Recent studies have focused on positive effects on the immune system. Bee pollen has attracted the attention of researchers because of its phenolic compounds and flavonoids. It was reported that bee pollen increases the immunoglobulin level (Oliveira *et al*., 2013; Fazayeli-Rad *et al*., 2015), with resulting increases in immunity (Kieliszek *et al.*, 2018; Bobiş *et al*., 2010). Feeding bee pollen has also been reported to increase white blood cell counts and lymphoid organ weights (Farag & El-Rayes, 2016). However, the results from supplementation with bee pollen have been mixed. Some studies found that the addition of bee pollen to the quail diet did not affect their performance and carcass quality (Sarıkaya *et al*., 2018; Attia *et al*., 2014), whereas other studies documented improvements in performance, carcass quality, and serum constituents (Farag-Soha & El-Rayes, 2016; İlçeli & Yıldız, 2021). Decreased calcium absorption and increased phosphorus absorption were also noted (Ivana *et al*., 2018). Thus, previous studies, taken together, provided clarity about the benefit or lack of it from supplementation of poultry with bee pollen and

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there were no recommendations on the amount that would be optimal. Therefore, this study aimed to evaluate the influence of dietary supplementation of bee pollen on performance, carcass, meat quality, and serum constituents in Japanese quail.

Material and Methods

During the study, all procedures were followed according to the criteria specified by the National Institute of Health Guide for the Care and Use of Laboratory Animals. A total of 160 one-day-old quail chicks were used. These chicks were randomly assigned to four treatments, each consisting of 40 chicks. These groups were further divided into four replicates of ten birds. The feeding period lasted 42 days. The basal diet was formulated as prescribed in NRC (1994) to provide 24% crude protein and 2900 kcal ME/kg (Table 1). The treatments were applied to the basal diet with the addition of the bee pollen at 0, 2.5, 5.0, and 10.0 g/kg of the feed. Feed and water were provided ad libitum throughout the experiment.

Table 1 Basal diet for growing Japanese quail and its nutrient composition

Ingredients	%	Nutrient	Amount
Yellow corn	47.95	Crude protein, %	24.07
Soybean meal (44% crude protein)	44.50	Metabolic energy, kcal/kg	2909
Sunflower oil	4.40	Calcium, %	0.85
Limestone	1.00	Available phosphorus, %	0.40
Dicalcium phosphate	1.20	Lysine, %	1.34
Salt	0.30	Methionine, %	0.52
Vitamin-mineral premix[1]	0.25	Cysteine, %	0.31
L-lysine	0.20	Methionine + cysteine	0.83
DL-methionine	0.20		

[1]1 kg premix contained vitamin A: 8.800 IU, vitamin D3: 2.200 IU, vitamin E: 11 mg, nicotinic acid 44 mg, calcium D-pantothenate: 8.8 mg, riboflavin: 4.4 mg, thiamine: 2.5 mg, vitamin B12: 6.6 mg, folic acid: 1 mg, D-biotin: 0.11 mg, choline: 220 mg, manganese: 80 mg, iron: 60 mg, copper: 5 mg, zinc: 60 mg, cobalt: 0.20 mg, iodine: 1 mg, selenium: 0.15 mg

Each replicate was placed in a cage measuring 40 cm x 40 cm. Light was provided continuously for the first three days. After that, a lighting regime of 23 hours of light and 1 hour of darkness was used. The temperature was set at 33 °C on the first day and decreased by 3 °C per week until 21 °C was reached.

The chicks were weighed at same time of day at the beginning and end of the experiment. Each replicate was fed as a group, with the feed being weighed daily. The remaining feed was subtracted from the feed at the end of the experiment. Live weight gain and the feed conversion ratio were calculated from these measurements. The number of birds that died was recorded each week and a weekly mortality rate was calculated from these data.

After being fed for 42 days, two birds (1 female, 1 male) were randomly selected from each replicate and slaughtered. Slaughter was carried out in an area close to where the birds had been fed. Thus, any possible effects of transportation stress were minimized. The selected quails were decapitated, and blood samples (5 ml blood sample) were collected into sterile tubes with anticoagulant. The weights of the thigh + drumstick, breast, liver, heart, and follicle were recorded, and these data were transformed to percentages of live weight.

The cross-sectional surface colour intensities (*L*, a** and *b**) of breast and thigh meats from the post-slaughter carcass were measured with a Minolta colourimeter (CR-200, Minolta Co., Osaka, Japan). The lightness (*L**), redness (*a**), and yellowness (*b**) values were based on the specifications of the International Commission on Illumination according to a three-dimensional colour scale: *L** = 0 = black, *L** = 100 = white; *a** = +60 = red, *a** = -60 =green; and *b** = +60 = yellow, *b** = -60 = blue.

The blood samples were centrifuged at 3000 rpm for 15 minutes to ensure the separation of the serum. An automatic enzyme analyser (Roche Cobas 6000 C501, Roche Diagnostics, Regensburg, Germany) was used to determine the levels of glucose, cholesterol, triglyceride, total protein, calcium,

phosphorus, magnesium, potassium, sodium, chlorine, and of immunoglobulin A and G (IgA and IgG) in the serum.

Data were analysed using one-way analysis of variance. If the *P*-value for treatment effects was less than 0.5, Duncan's multiple comparison test was used to determine the differences between the treatment means (SPSS, version 24.0, IBM Corp., Armonk, New York, USA).

Results and Discussion

There were no significant differences between the treatments in the measures of live performance (Table 2). There was a tendency for the birds fed bee pollen to consume more feed compared with the control group. These results were not similar to earlier studies in which it was reported that at least one of the performance measures improved with the addition of bee pollen to the diet in growing birds. For example, adding bee pollen was effective in increasing live weight (Hosseini *et al*., 2016; El-Medany *et al.,* 2017), reducing feed consumption (Seven *et al*., 2011; Farag-Soha & El-Rayes, 2016), and improving feed efficiency (İlçeli & Yıldız, 2021). These differences between the results might be because of the bio-active substances in the bee pollen as a result of variations in the flora being pollenated by the bees.

Table 2 Effects of adding bee pollen to the diet of growing quail on their weight gain and feed intake

Treatment[1]	Initial weight, g	Final weight, g	Weight gain, g	Feed intake, g	Feed conversion ratio
0.0	10.4	188.2	177.8	706.5	3.98
2.5	9.9	199.3	189.4	758.3	4.01
5.0	10.3	195.6	185.3	756.4	4.08
10.0	10.3	197.4	187.1	740.5	3.96
SE	0.21	4.55	4.56	12.77	0.10
P-value	0.41	0.37	0.35	0.07	0.82

[1]Treatment is designated as grams of bee pollen added to each kg of feed

No significant differences between treatments were detected for the percentages of skinless carcass, thigh and drumstick, breast, liver, and heart (Table 3). Similarly, Haščík *et al.* (2015b), El-Medany *et al*. (2017) and Haščík *et al*. (2019) reported that the effects of bee pollen supplementation on carcass weight were not significant. However, Farag-Soha & El-Rayes (2016) found that the percentage of the carcass was affected significantly by adding bee pollen. There seems to be a contradiction among studies, which might have been the result of differences in the levels of nutrients provided to the animals relative to their requirements. Birds that were provided a higher level of nutrition showed clearer effects on their performance.

Table 3 Effects of adding bee pollen to the diet of growing quail on their carcass characteristics and organ weights (% of live weight)

Treatment[1]	Carcass	Breast	Thigh+ Drumstick	Liver	Heart	Follicle
0.0	57.9	37.5	35.8	4.22	1.43	4.90^{c}
2.5	58.6	37.5	36.8	4.21	1.39	6.18^{bc}
5.0	57.8	38.5	35.8	3.81	1.41	6.75^{ab}
10.0	57.2	37.5	36.4	3.88	1.36	7.57^{a}
SE	0.94	0.85	0.49	0.22	0.04	0.32
P-value	0.81	0.81	0.43	0.42	0.62	0.05

[a,b,c] Within a column, means followed by a common superscript were not different with probability *P* =0.05
[1] treatment is designated as grams of bee pollen added to each kg of feed

The effect of treatment on follicle weight was significant (P <0.05), and the addition of bee pollen to the diet incrementally increased the relative weight of the follicle (Table 3). Follicle growth had not been measured previously, but it appeared that supplementation with bee pollen might affect the reproduction of the hens positively. The active substances in bee pollen may have increased the release of oestrogen. Kolesarova *et al*. (2013) showed that oestradiol secretion was increased after bee pollen treatment at 5 g/kg and bee pollen was a potent regulator of rat ovarian functions. Additionally, the effects of bee pollen on plasma hormones might have been because of the vitamins, minerals, phospholipids, and copper it contains, with these nutrients playing a role in reproductive performance (Attia *et al.*, 2010).

Because poultry cannot synthesize carotenoids, they must obtain them from their diet (Hu *et al.,* 2012). Thus, the colour of a bird's skin depends mainly on the total amount of carotenoids in diet, especially xanthophylls and their absorption and accumulation in the skin and subcutaneous fat. The breast and thigh meat colour values, *L*, a**, and *b** are shown in Table 4. Bee pollen supplementation affected the *L** value of the breast meat (P <0.05) and the *a** value of the thigh meat (P <0.05). However, it did not otherwise affect the colour of the breast or thigh meat. Compared with the control, the addition of 5 g/kg bee pollen made the breast meat significantly darker and the thigh meat significantly less red. Haščík *et al*. (2015a) observed that thigh and breast meat *a** values were different in broilers that were supplemented with bee pollen. Prakatur *et al*. (2020) observed no significant effects on *L** and *a** values for breast meat, which could be attributed to the effects of supplementation with bee pollen. However, Prakatur *et al*. (2020) reported that the supplementation effect on *b** value was significant (P <0.01). Mancini and Hunt **(**2005) explained that the *L** and *a** measurements could easily be applied to muscle colour, whereas the colour spectrum represented by *b** is not characteristic of meat. Wideman *et al*. (2016) reported that the haemoglobin and myoglobin contents were the higher contributors to the colour of poultry meat and observed that consumers had a clear preference for lighter coloured poultry meat.

Table 4 Effects of adding bee pollen to the diet of growing quail on the colour pf breast and thigh meat from 42-day-old Japanese quail

Treatment[1]	Breast			Thigh		
	*L**	*a**	*b**	*L**	*a**	*b**
0.0	39.3[b]	5.52	5.13	37.91	5.27[a]	3.28
2.5	41.9[ab]	5.45	4.94	38.63	4.47[ab]	1.99
5.0	43.3[a]	5.22	5.25	39.27	3.57[b]	1.32
10.0	42.2[ab]	5.76	5.64	38.39	4.11[ab]	2.60
SE	0.83	0.29	0.25	1.05	0.36	0.62
P-value	0.03	0.65	0.30	0.83	0.04	0.20

*L**: lightness, *a**: redness, *b**: yellowness
[a,b] Within a column, means followed by a common superscript were not different with probability P =0.05
[1] treatment is designated as grams of bee pollen added to each kg of feed

In this study, supplemental bee pollen did not affect serum glucose, triglyceride, cholesterol, and total protein levels in growing quails (Table 5). In other studies, the addition of bee pollen to the diet decreased serum cholesterol (Farag-Soha & El-Rayes, 2016; Demir & Kaya 2020) and triglyceride (Ivana *et al*., 2018; Zeedan *et al*., 2017; Demir & Kaya, 2020*)* levels. Conversely, increased levels of serum glucose (Ivana *et al*., 2018) and total protein (Farag-Soha & El-Rayes, 2016; Zeedan *et al*., 2017) were observed in poultry supplemented with bee pollen.

The addition of various levels of bee pollen to the diet did not affect the serum mineral profile of the growing quail (Table 6). Ivana *et al.* (2018) found that supplying broiler chickens with 20 g/kg bee pollen affected the levels of calcium, phosphorus, sodium, and chloride in their serum significantly. However, the levels of magnesium and potassium were unaffected. Demir and Kaya (2020) reported that supplementation of laying hens with bee pollen had no significant effect on serum calcium concentration. However, Demir and Kaya (2020) found that the serum phosphorus and magnesium levels increased linearly with the level of supplementation.

Table 5 Effects of adding bee pollen to the diet of growing quail on serum glucose, triglyceride, cholesterol, and protein levels of 42-day-old Japanese quail

Treatment[1]	Glucose, mg/dL	Triglyceride, mg/dL	Cholesterol, mg/dL	Total protein, g/dL
0.0	308.1	585	163.4	3.22
2.5	306.3	486	155.3	3.20
5.0	312.0	810	152.7	3.55
10.0	317.5	479	161.8	3.17
SE	5.89	160.9	9.06	0.25
P-value	0.57	0.46	0.81	0.69

[1] treatment is designated as grams of bee pollen added to each kg of feed

Table 6 Effects of adding bee pollen to the diet of growing quail on the serum mineral profile of 42-day-old Japanese quail

Treatment[1]	Calcium, mg/dL	Phosphorus, mg/dL	Magnesium, mg/dL	Potassium, mmol/L	Sodium, mmol/L	Chlorine, mmol/L
0.0	14.8	7.06	3.62	2.87	148.7	109.2
2.5	15.2	7.26	3.42	2.76	148.1	108.0
5.0	14.8	7.03	3.40	2.25	147.0	108.1
10.0	14.2	6.46	3.77	2.75	148.3	108.6
SE	1.01	0.40	0.21	0.23	0.71	0.74
P-value	0.93	0.69	0.66	0.34	0.42	0.60

[1] Treatment is designated as grams of bee pollen added to each kg of feed

The findings for IgA and IgG levels of the 42-day-old birds are given in Table 7. In this study, IgA and IgG levels differed ($P \leq 0.01$) in response to the treatments. The IgA level was elevated in response to supplementation with bee pollen, regardless of the level that was provided. The treatment effects on IgG were less sensitive, with the level of IgG being elevated when the bee pollen was fed at a level of 10 g/kg of the ration compared with 5 g/kg. However, the unsupplemented control and the group supplemented at 5 g/kg were not different from either of the other treatments.

Table 7 Effect of adding bee pollen to the diet on serum IgA and IgG values of growing quails

Treatment[1]	Immunoglobulin A, mg/dL	Immunoglobulin G, mg/dL
0.0	7.4^{b}	30.0^{ab}
2.5	10.9^{a}	27.5^{b}
5.0	11.6^{a}	30.9^{ab}
10.0	10.8^{a}	35.2^{a}
SE	0.62	1.30
P-value	<0.01	0.01

[1] Treatment is designated as grams of bee pollen added to each kg of feed
[a,b] Within a column, means followed by a common superscript were not different with probability $P = 0.05$

Zeedan *et al*. (2017) reported that bee pollen supplementation affected the IgA and IgG levels significantly in male rabbits. Although the species difference must be considered when comparing the results

of this study with those of Zeedan *et al*. (2017), the agreement is notable. Oliveira *et al*. (2013) reported that bee pollen was rich in nutrients that aid in cell differentiation and proliferation and stimulate immunity. Wang *et al*. (2005) stated polysaccharides in bee pollen could affect T lymphocyte production and that various dosages of broken wall bee pollen could improve the antibody titre to virus and bacterial antigens to varying degrees. In the present study, the effects on IgA and IgG values that were observed might have been because the bee pollen produced changes in the immune system that activated its function or overstimulated it. Bee pollen could also be an allergen, and this possibility was not assessed in this study.

Conclusions

These results bring more clarity to the effects of supplementation with bee pollen on the productivity and health of Japanese quail. Further investigation is warranted. The effects of supplementation with bee pollen on the immune system appear promising.

Authors' Contributions

BS was solely responsible for the reported research.

Conflict of Interest Declaration

The author has no competing interests.

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