Effects of propolis and pollen supplementations on growth performance and body components of Japanese quails (Coturnix coturnix japonica)

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Abstract

The present study was carried out to investigate the effects of honey bee propolis and pollen supplementation on growth performance and body components in quail (Coturnix coturnix japonica). Two experiments were conducted. In the first experiment, propolis ethanolic extract (30%, PEE) was supplemented in diets at levels of 0, 5 and 10 ml PEE kg–1 while in the second experiment, pollen was supplemented in diets at four levels (0, 5, 10 and 20 g pollen kg–1). In both experiments, chicks were fed with diet containing 240 g crude protein and 3100 kcal ME per kg diet. During the experimental period, body weight, feed consumption and feed efficiency were determined weekly. At the end of the experiments, 3 female and 3 male quail from each subgroup were killed humanely to determine body components.

Experimental results showed that supplementation of PEE and pollen did not significantly affect body weight gain, feed efficiency or body components ($P > 0.05$). It was concluded that propolis and pollen had no effect at the levels investigated on performance and body components of quail.

Key words: Japanese quail, nutrition, propolis, pollen, growth

Introduction

Antibiotics have been added to poultry feed to improvegrowth performance, to stabilize intestinal microflora andto prevent infection by specific pathogenic microorganisms. However, concerns about antimicrobial resistance have existed for nearly as long, and recent concerns regarding the prevalence of antibiotic-resistant infections in humans have raised the controversy to new heights (Revington, 2002). For these reasons antibiotic growth promoters for poultry diets have been banned for use in the European Union and pressure from consumer groups and major poultry buyers has threatened their removal from diets in the US. Therefore, studies on alternate products that can result in promotion of growth, improved feed utilization,

and maintenance of gut health are taking place (Zhang et al., 2005). For this reason, the natural material propolis is being investigated.

Propolis (bee glue) is a natural resinous hive product collected by bees from plants, particularly from flowers and leaf buds. Bees use propolis to cover the inside of the hive and mix it with bees' wax when building combs to protect the colony and larvae from pathogenic microorganisms (Krell, 1996). Propolis contains a variety of chemical compounds such as polyphenols (flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, alcohols, and ketones), terpenoids, steroids, amino acids and inorganic compounds (Dimov et al., 1991; Moreno etal., 2000).

Many biological properties, including antibacterial (Velikova et al., 2000; Pepeljnjak et al., 1985), antifungal (Dimov et al., 1991; Murad et al., 2002), antiviral (Amoros et al., 1994), antioxidant (Isla et al., 2001), hepatoprotective (Gonzales et al., 1995), and immunostimulating (Dımov et al., 1991) activities of propolis have been reported. Now that antibiotic usage in animal nutrition is banned, propolis can be used to replace antibiotics. The antibiotic-like properties of propolis have been demonstrated by previous investigators. Propolis preparations show in vitro anti-microbial activity mainly against Gram-positive (Staphylococci and Strepthococci spp.) and Gram-negative bacteria (E. coli, K. pneumoniae, P. Vulgaris and P. aeruginosa), Helicobacter pylori, protozoa (T. cruzi), fungi (Candida albicans) and viruses (HIV, Herpes viruses or influenza viruses) (Scheller, 1990). Modern herbalists recommend propolis for human use in medicine because of its antibacterial, antifungal, antiviral, hepatoprotective and anti-inflammatory properties to increase the body's natural resistance to infections and to treat gastroduodenal ulcers (Castaldo and Capasso, 2002).

The pollen collected by Apis mellifera L. from different plant sources generally contains 40% protein, indispensable amino acids, low fat and high minerals (Sahinler, 2000). The chemical composition of pollen is given in Table 1 (Schmidt, 1997). In an excellent review, Schmidt and Buchmann (1992) compared the average protein, fat, mineral and vitamin content of pollen with other basic foods. Pollen was richer in most ingredients when compared on a weight or calorie content basis than foods such as beef, fried chicken, baked beans, whole wheat bread, apple, raw cabbage and tomatoes. While comparable in protein and mineral content with beef and beans, pollen contains more than ten times the thiamin and riboflavin or several times the niacin content. Pollen is usually consumed in such small quantities that the daily requirements for vitamins, proteins and minerals cannot be taken up through the consumption of pollen alone (Krell, 1996). Pollen may be used to strengthen the

immune system, to reduce the effect of radiation and retard aging because of its antioxidant and flavonoid contents (Geyman, 1994).

Table 1. Chemical composition of the pollen

Component	Rate	Component	Rate
Energy, kcal/kg	2.46	Nickel, ppm	4.50
Protein, %	23.7	Thiamin, ppm	9.40
Carbohydrate, %	27	Niacin, ppm	157
Lipid, %	4.8	Riboflavin, ppm	18.6
Phosphorus, %	0.530	Pyridoxine, ppm	9
Potassium, %	0.580	Pantothenate, ppm	28
Sodium, %	0.044	Folic acid, ppm	5.20
Calcium, %	0.225	Biotin, ppm	0.32
Magnesium, %	0.148	Vitamin C, ppm	350
Zinc, ppm	87	Carotenes, ppm	95
Copper, ppm	14	Vitamin, ppm	14
Iron, ppm	140		

Source: Schmidt, 1997.

There have been limited studies on the effects of propolis on the growth performance of poultry species. In these studies propolis did not affect the growth performance of poultry, possibly because of the use of low concentrations of propolis (Acıkgoz et al., 2005; Sahin et al., 2003). Higher ethanolic concentrations of propolis may increase the growth performance in quail. The insufficient findings about propolis and lack of availability of work on pollen in poultry encouraged us to conduct the current study in which dietary propolis was used as a substitute for antibiotic growth promoters and pollen as a performance enhancer in Japanese quail.

Material and Methods

Animals, diets and feeding treatments

This study consisted of two experiments, one using propolis and the other using pollen. In the propolis experiment, one hundred and eighty day-old Japanese quail (Coturnix coturnix japonica) were used. Quail chicks were weighed and divided equally into three groups with equal sex ratio (10 males and 10 females) and uniform body weight (9.12 ± 0.060 g). In the pollen experiment, one hundred and eighty day-old Japanese quail (Coturnix coturnix japonica) were used. Quail chicks were weighed and divided equally into four groups with uniform body weight (8.29 ± 0.039 g). For both experiments, chicks were sub-divided into three replicate groups within each treatment group and transferred to growing cages sized 50 × 50 × 17 cm. In the pollen experiment each replicate included 8 males and 7 females.

The same isocaloric and isonitrogenous experimental diet was formulated to meet the nutrient requirements for quail chicks (NRC, 1994) for both experiments. The ingredients and composition of the diet (240 g crude protein and 3100 kcal ME kg–1) are presented in Table 2.

Table 2. Feed ingredients and composition of experimental diet

Ingredients	kg	Nutrient content	
Corn	480	Dry matter; %	88.71
Soyabean meal	200	Crude protein, %	24.20
Corn gluten meal	140	Crude fat, %	5.75
Razmol	100	Crude fibre, %	3.31
Fish meal	30	ME (kcal/kg)	3040
Vegatable oil	25	Ca, %	0.84
Vitamin premix*	2.5	Available P, %	0.27
Mineral premix**	1.5	P, %	0.52
DCP	3	Lysine, %	1.32
Limestone	15	Methionine+Cystine, %	0.92
L-Lysine	2		
Salt	1		
Total	1000		

*Each kg of vitamin premix contains 4.800.000 IU Vitamin A; 600.000 IU Vitamin D_3; 12.000 mg Vitamin E; 2.000 mg Vitamin K_3; 1.200 mg Vitamin B_1; 2.400 mg Vitamin B_2; 2.000 mg Vitamin B_6; 12 mg Vitamin B_{12}; 16.000 mg Nicotinamid; 4.000 mg Ca-D-Panthothenate; 300 mg Folic Acid, 30 mg D-Biotin; 150.000 mg Choline Chloride.

** Each kg of mineral premix contains 80.000 mg Mn; 80.000 mg Fe; 60.000 mg Zn; 8.000 mg Cu; 500 mg I; 200 mg Co, 150 mg Se.

In the propolis experiment, each kg of the diet was sprayed and mixed with 0 ml (group 1), 5 ml (group 2) and 10 ml (group 3) propolis ethanolic extract (PEE), while in the pollen experiment each kg of diet was mixed with 0 g (group 1), 5 g (group 2), 10 g (group 3) and 20 g (group 4) pollen powder. During the experimental period, the quails were maintained on a 24-h constant lighting schedule and allowed access to feed and tap water ad-libitum until slaughter at 35 d of age.

Growth parameters measured

During the experimental period, the quails were individually weighed and feed consumption per pen was recorded weekly. The uneaten feed was discarded and replaced with fresh feed daily and feed efficiencies were calculated weekly. Mortality was recorded as it occurred and percentage mortality was determined at the end of the study. At the end of the experiments, 3 female and 3 male birds of average body weight for both sexes in each replicate group were slaughtered to determine carcass characteristics. Carcass yield was calculated from eviscerated weight and live weight. The internal organ weights are given as percentage of carcass weight.

Samples and biochemical analysis of propolis

Current propolis solid yield and pollen grains were collected from bee colonies on the MKU Research Farm located in Hatay province, Turkey, in 2005. Hatay is located between latitude 36° north and longitude 36° east in the Eastern Mediterranean region where climatic conditions are hot and dry in summer, and warm and rainy in winter. Common species of flora in the Hatay region include Medicago orbicularis (flat-podded medick, button clover), Medicago rotata (well medick), Trifolium spumosum (sea clover), Lathyrus sativus L. (grasspea, chickling pea), Coronilla varia (trailing crown vetch), Lotus spp. (bird's foot), Pisum arvense (field pea), Adonis spp. (pheasant's eye, autumn Adonis), Anagalis arvensis (scarlet pimpernel, blue pimpernel), Hordeum bulbesum (bulbous wild barley), Aegilops ovata (little goatgrass), Convovulus sp. (field bindweed, creeping jenny), Anthemis sp. (Anatolian mountain chamomile), Salvia multicaulis Vahl. (Shell flower sage), Ferula communis (Umbelliferae), Medicago sativa L (alfalfa, lucerne), and Detroselinum sativum (parsley) (Sahinler et al., 2004). Following collection, propolis was kept desiccated in the dark until processing. A 30% propolis tincture was prepared by adding 600 g propolis to 1400 ml 70% ethanol (w/v). This was mixed and kept in a glass container, shaken twice daily, filtered after one week and kept at 4°C until it was used (Krell, 1996). Propolis was analysed chromatically in a GC-MS (Hewlett Packard Gas Chromatograph 6890 Series plus linked to a Hewlett Packard 6890 Mass Spectrometer) system for the biochemical analysis. A capillary column (25 μm thick, 0.25 mm diameter, 30 m long) and helium carrier gas (31 ml/min linear velocity, 20:1 split ratio, and 230°C temperature) were used in the GC-MS system. The volatile substances (terpenoids) were analyzed from the etheric extract (this extract was prepared in the same way as the ethanolic extract, but using ether); the other components were analyzed from the ethanol extracts of propolis. The biochemical contents of propolis for both ethanolic and etheric elutions are given in Table 3.

Statistical Analysis

The data obtained in the experiments were analysed statistically using the One Way ANOVA procedure of SPSS (Windows Version of SPSS, release 10.01) with Duncan's Multiple Range Test to identify the significant differences between the means.

Results and Discussion

In the present study propolis had high concentrations of aromatic acids such as benzyl cinnamate, methyl cinnamate, caffeic acid, cinnamyl cinnamate and cinnamoylglycine (Table

3). These aromatic compounds are responsible for the antibacterial, antifungal, antiviral, antiinflammatory and anticancer properties of propolis (Yamomoto, 1997; Vilanueva et al., 1970; Bankova et al., 1983; Bankova et al., 2000; Velikova et al., 2001; Sahinler et al., 2002; Sahinler and Kaftanoglu, 2005).

Table 3. Biochemical composition of etheric and ethanolic extract of propolis

Substances	Ethanol Extracts % Area	Etheric Extracts % Area	Substances	Ethanol Extracts % Area	Etheric Extracts % Area
Aromatic acids			**Hydrocarbons**		
Benzyl cinnamate[5]	3.25	-	Nonacosane[1,5]	0.25	-
Methyl cinnamate[5]	1.25	-	Triacontane[5]	0.14	-
Caffeic acid[1,3,4,5]	3.93	-	Heneicosane[5]	0.23	-
Cinnamyl cinnamate[5]	5.99	-	Triacosane[5]	1.18	-
Cinnamoylglcine [5]	0.83	-	Hexacosane[1, 5]	0.22	-
Terpenes			Ketonlar		-
Alpha-Pinene[25]	0.53	-	Pentadecanone	0.81	-
Indolin, 2- methylene [5]	1.25	-	**Fatty acids**		
Cyercene[5]	3.23	-	13-Tetradecanol	0.24	-
1S-Cis-Calamene[5]	0.74	-	Hexadecanoic acid[1,2]	1.95	4.14
Alpha-Copaene[15]	2.23	-	9-Octadecanoic acid[1]	2.22	10.81
Beta-Maaliene[5]	1.26	-	Docosanoic acid[1]	1.34	1.82
Alpha-Elemene[5]	2.14	-	Tetracosanoic acid[1]	1.88	-
Beta-Eudesmol[5]	7.63	-	Hexacosanoic acid	4.29	-
Alpha-Eudesmol[5]	1.39	-	Octacosanoic acid[1]	2.24	-
Alpha Bisabolol[5]	2.15	-	Triacontanoic acid	1.74	-
1-Naphthalenol	-	0.93	Butanedioic acid	1.22	0.58
5.alpha-androstan-16-One	-	0.55	Octadecanoic acid	3.18	1.24
Geranyl acetate	0.43	-	9,12-Octadecanoic acid	2.81	1.47
Calarene	0.85	-	9,12,15-Octadecatrienoic acid	1.25	0.94
Aldehydes			Eicosanoic acid	1.65	0.57
Decyl Aldehyde[5]	0.05	-	Tetracosanoic acid	3.38	9.94

The results obtained in the propolis experiment are summarized in Table 4. PEE supplementation did not affect weight gain and feed intake of quail chicks. However, PEE affected weight gain for a period of 1–21 days (Figure 1). A similar finding was reported by Biavatti et al. (2003) who found that Alternanthera brasiliana and propolis extracts increased body weight gain from 14 to 21 days. Increased dietary PEE supplementation (0, 5 and 10 ml

PEE kg–1) tended to improve feed efficiency, but not statistically. These findings are in agreement with the results of Sahin et al. (2003) who indicated that addition of propolis ethanolic extract to quail diets did not affect body weight gain, feed intake or feed efficiency. Similarly, Acikgoz et al. (2005) indicated that propolis supplementation at levels of 500 and 2000 ppm per kg diet did not improve the performance of male broilers. Controversially, Denli et al. (2005) reported that addition of propolis powder at 0.5, 1 and 1.5 g per kg diet increased the growth parameters of quail chicks. Also, Ghisalberti (1979) reported that supplementation with propolis in broiler diets at a level of 500 ppm increased body weight gain by 20%.

Table 4. Effects of PEE on growth performance and body components of quail

Parameter per bird	Supplemental PEE (ml kg^{-1})			
	0	5	10	SEM
Body weight gain, g	223	226	224	1.39
Feed intake, g	609	615	603	7.47
Feed conversion ratio (g feed : g gain)	2.73	2.72	2.70	0.027
Slaughter weight, g	243	249	247	1.80
Carcass weight, g	178	184	183	1.48
Carcass yield, %	73.0	74.0	74.1	0.294
Liver weight, %	2.67	2.59	2.86	0.065
Gizzard weight, %	3.12	2.95	2.94	0.065
Heart weight, %	1.22	1.25	1.24	0.024

SEM: Pooled standard error of the mean

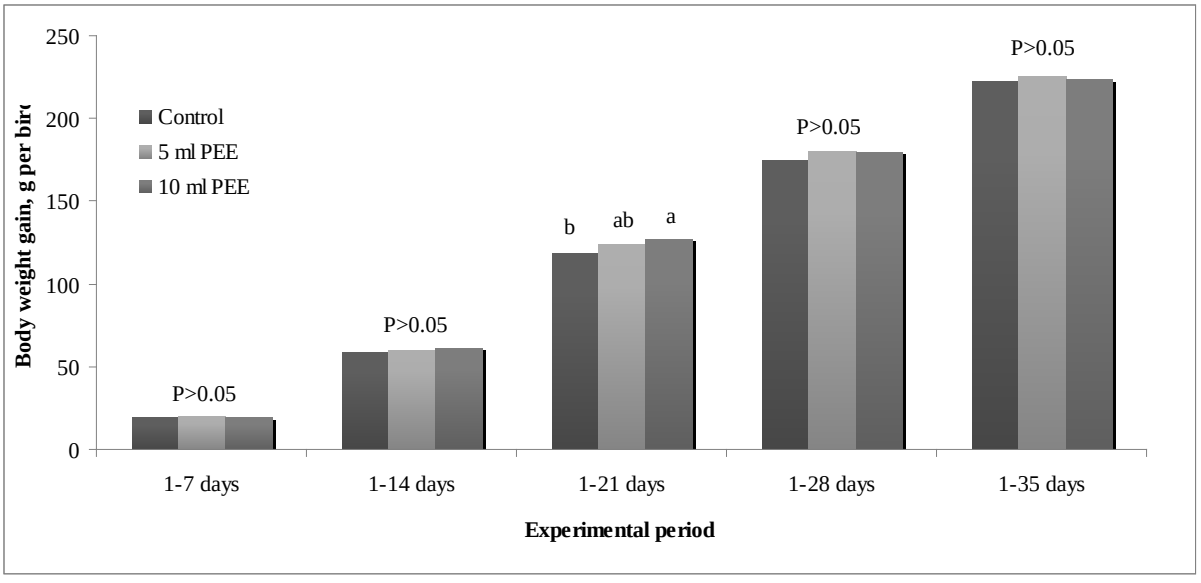

Figure 1. The effect of propolis supplementation on body weight gain of quail chicks

Carcass yield and internal organ weight, such as liver, gizzard and heart, were not affected by PEE supplementation. There were no significant differences ($P > 0.05$) between groups for any of the measurements. Our results are in line with the findings of Denli et al.

(2005) who indicated that addition of propolis and flavomycine to quail diets did not affect carcass characteristics. Similarly, Sahin et al. (2003) found that the addition of PEE (5%) at a level of 0, 6, or 12 ml PEE kg–1 did not affect carcass characteristics except carcass yield.

During the experimental period mortality rates were 5.33% for control groups and 2.66% for the treatment groups.

The ethanol extract of propolis was not effective in this study. This may be attributed to the lower dose of propolis and to the fact that birds were kept in hygienic conditions in cages where there were no challenging factors affecting the gastrointestinal health of the birds. Feed intake did not decrease in PEE birds. It is likely that the organoleptic properties of propolis at the doses used are acceptable for the birds.

The results obtained in the pollen experiment are summarized in Table 5. Body weight gain tended to increase with increasing levels of pollen supplementation (0, 5, 10 and 20 g pollen powder per kg diet) to the quail diet, but without statistical significance ($P > 0.05$). Figure 2 also shows the significant effects of pollen supplementation on cumulative body gain (1–28 days, $P < 0.05$) during the experimental period. Feed intake was higher when pollen was included in the diets. The addition of pollen at 5, 10 and 20 g/kg–1 in the diet significantly increased feed consumption by quail chicks ($P < 0.05$). Cumulative feed intake increased with increasing levels of pollen supplementation. However, feed efficiency decreased with increasing levels of supplementation, although the differences were not significant ($P > 0.05$). Higher feed intake and the non-significantly reduced feed conversion ratio may be the result of a depression of digestibility by the added pollen.

Table 5. The effects of pollen supplementation on growth performance and body components of quail

Parameter per bird	Supplemental pollen (g/kg^{-1})				
	0	5	10	20	SEM
Body weight gain, g	218	225	225	225	1.58
Feed intake, g	579^{b}	599^{ab}	614^{a}	622^{a}	5.97
Feed conversion ratio (g feed : g gain)	2.66	2.68	2.74	2.76	0.02
Slaughter weight, g	235	240	237	238	1.50
Carcass weight, g	175	180	176	178	1.29
Carcass yield, %	74.6	75.0	74.1	74.6	0.287
Liver weight, %	3.07	3.46	3.51	3.18	0.084
Gizzard weight, %	3.25	3.17	3.30	3.20	0.069
Heart weight, %	1.29	1.25	1.41	1.22	0.027

SEM: Pooled standard error of the mean

* Means within row with different superscripts differ significantly ($P<0.05$).

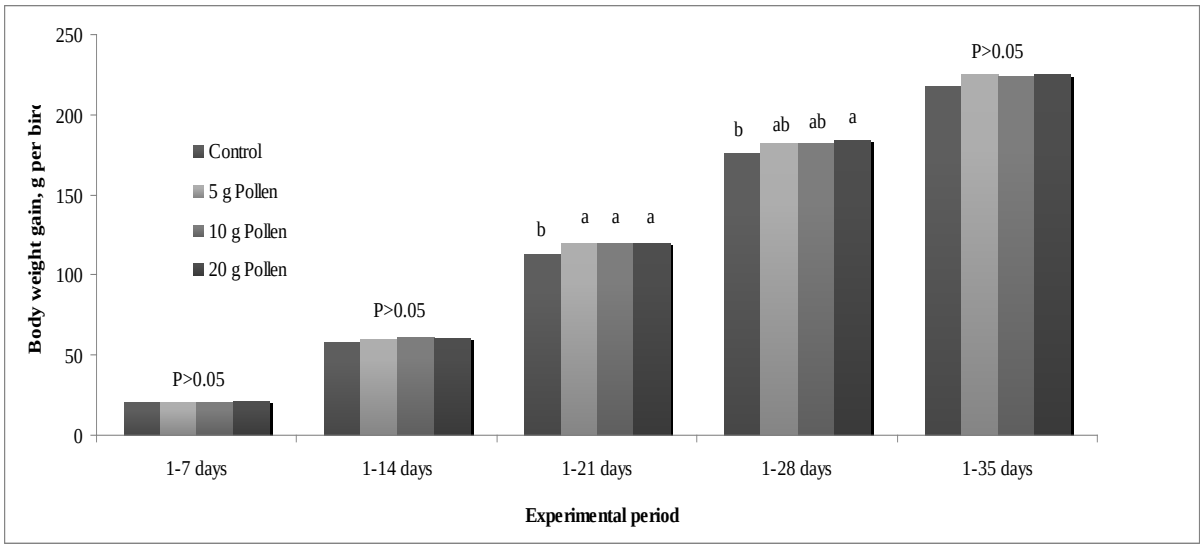

Figure 2. The effect of pollen supplementation on body weight gain of quail chicks

There were no significant differences between groups for any of the carcass characteristics measured in the pollen experiment ($P > 0.05$).

In conclusion, under the conditions investigated, PEE (30%) and pollen supplementation did not result in any significant improvement in growth performance and body components of quail. Therefore, propolis and pollen cannot be recommended as a growth promoter in quail production. However, propolis may show advantageous effects under poor hygienic conditions. Therefore, more extended research should be planned for determining the antiviral, antibacterial and antimicrobial effects of propolis on the immune system in poorer hygienic conditions.

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