

Effect of Dietary Addition of Turkish Propolis on the Growth Performance, Carcass Characteristics and Serum Variables of Quail (*Coturnix coturnix japonica*)

M. Denli*, S. Cankaya, S. Silici¹, F. Okan and A. N. Uluocak

Department of Animal Science, Çukurova University, Adana 01330, Turkey

ABSTRACT : We examined the effect of dietary Turkish propolis and flavomycin on growth performance, carcass characteristics, internal organ weights and some serum variables in quail (*Coturnix coturnix japonica*) birds. One hundred and fifty day-old quails were randomly divided into five groups, with ten replicate pens per treatment and three birds per pen. One group received the basal diet (antibiotic-free), the control. The flavomycin at 10 mg/kg diet and propolis at 0.5, 1 and 1.5 g/kg diet were added to the basal diet. Body weight gain, feed consumption and feed efficiency were determined weekly. Carcass characteristics, internal organ weights and serum variables were determined at the end of the study (35 day). The results showed that body weight gain, feed efficiency and carcass weight were improved significantly ($p < 0.01$) when compared to control group for birds fed diets containing propolis and flavomycin between 14 to 35 days. The addition of 1 g/kg propolis to the diet resulted in significantly ($p < 0.01$) better-feed efficiency as compared to control and other treatment groups. There were no significant differences in carcass yield, abdominal fat, liver gizzard, proventriculus and intestinal weight and intestinal pH among the groups. In addition, serum ALP, total protein, uric acid, cholesterol and triglyceride were not influenced by the any supplementation. However, birds fed with propolis tended to have higher serum HDL and lower level than birds fed the control diet. In conclusion, supplementation of propolis and flavomycin during the growth period showed similar effects on growth performance in quail. Therefore, it can serve as a natural substitute for antibiotics in poultry diets. (*Asian-Aust. J. Anim. Sci.* 2005. Vol 18, No. 6 : 848-854)

Key Words : Propolis, Flavomycin, Growth Performance, Carcass Characteristics, Serum Variables, Quails

INTRODUCTION

Antibiotics have been used in animal agriculture as feed additives since shortly after their discovery. For a number of years antibiotics have been used extensively as growth promoters in animal feeds all over the world, especially in the poultry and pig production industries. The use of antibiotics in diet as growth promoters is aimed primarily at the improving of growth performance of poultry such as improved feed efficiency and increasing body weight gains in broiler production. However, concerns of antimicrobial resistance have existed for nearly as long, but concerns regarding the prevalence of antibiotic-resistant infections in human have raised the controversy to new heights (Revington, 2002). Due to these reasons the use of antibiotics has been limited in the European Union (EU). The ban of several antibiotics has caused a resurgence of infectious diseases and economic losses in the poultry industry. This has prompted many scientists and industry to search for alternatives to antibiotics.

One alternative may be incorporation of propolis into broiler diets. Propolis is a resinous material gathered by honeybees (*Apis mellifera caucasica*) from the buds and

bark of certain trees and plants. It contains a variety of substances including phenolic compounds such as flavonoids, aromatic acids and their derivatives, esters, alcohols and trace elements (Ghisalberti, 1979). Propolis is alleged to exhibit a broad spectrum of activities including antibacterial (Kujumgiev et al., 1999; Sforzin et al., 2000), antifungal (Ota et al., 2001; Sawaya et al., 2002), antiviral (Manolovan et al., 1985; Amaros et al., 1994), anti-inflammatory (Miyataka et al., 1997) local-anesthetic (Paintz and Metzner, 1979) antioxidant (Orhan et al., 1999) immunostimulating (Dimov et al., 1991) and cytostatic (Banskota et al., 2000) properties. Also, most studies have indicated that, propolis compounds such as quercetin, luteolin (flavonoids), artemillin-C, caffeic acid and caffeic acid phenyl ester exhibit antitumor effects (Verma et al., 1988; Deschner et al., 1991; Sud'ina et al., 1993; Matsuno et al., 1997). It has been shown that the main source of Turkish propolis is poplar bud exudates (Velikova et al., 2000) and that it has antibacterial, antifungal, antioxidant and antiinflammatory activity (Keskin et al., 2001; Kolonkaya et al., 2002; Silici and Kaftanoglu, 2003). Popova et al. (2005) stated that detailed study of the qualitative chemical differences between sample of poplar (Kayseri, Central Anatolia) and of mixed origin (Adana, Artvin and Erzurum) was performed by GC-MS and the Kayseri sample was confirmed to contain the typical poplar flavonoid aglycones, phenolic acids and esters.

Recently, propolis has also been extensively used in

* Corresponding Author: Muzaffer Denli. Tel: +90-322-3386813 (24), Fax: +90-322-3386576, E-mail: mdenli@cu.edu.tr

¹ Department of Animal Health, Erciyes University, Kayseri, 38039, Turkey.

Received September 26, 2004; Accepted January 15, 2005

food and beverages to improve health and prevent disease such as inflammation, heart disease, diabetes and cancer. Because of its wide range of biological activities and its use as a health food, there is a renewed interest in the composition of propolis.

Due to these properties, propolis was selected for use in this experiment. The aim of the present study was to examine the effects of the use of dietary propolis as a substitute for antibiotic growth promoters on growth performance and to determine whether propolis supplementation would affect carcass characteristics, internal organ properties and serum variables of quail birds.

MATERIALS AND METHODS

Animals, diets and feeding treatments

150 day-old Japanese quail (*Coturnix coturnix japonica*) were used in this experiment. Birds were weighed (9.2 g) and randomly assigned to five groups of 10 replicate pens (30×50 cm), containing 3 chicks using a completely randomized design (CRD). The diet (antibiotic-free) was formulated to meet the nutrient requirements of the broiler during the starter (0-35) period according to the National Research Council (NRC, 1994) recommendations. The ingredients and compositions of the diets are presented in Table 1. The five dietary treatments were: 1) Control, 2) 10 mg/kg feed flavomycin, 3) 0.5 g/kg feed propolis, 4) 1 g/kg feed propolis and 5) 1.5 g/kg feed propolis. During the experimental period (35 days), conventional brooding and management procedures were employed. The birds were exposed to continuous lighting and birds were fed and watered *ad libitum*. The experiment ended within 35 days.

Growth parameters measured

The body weights of the birds were individually weighed and feed consumptions per pen were recorded weekly, the uneaten feed discarded, and feed efficiencies were calculated. Mortality was recorded as it occurred and percentage mortality was determined at the end of study. On day 35 eight birds from each experimental group were weighed and slaughtered by cutting the jugular vein, exsanguinated, defeathered and eviscerated. Carcass yield was calculated from eviscerated weight and live weight. Abdominal fat weight was measured and calculated.

Determination of weight of organs

At the end of 35 days, 8 birds from each treatment were selected based on the average weight of the group and sacrificed. The intestine was separated from the rest of the gastrointestinal tract after it was removed from birds. Intestinal length and weight were recorded. The other organs, including liver and gizzard were also removed and weighed.

Table 1. Composition of basal diets during the experiment (%)

| Ingredients | 0-35 days |
|----------------------------------|-----------|
| Maize | 54.62 |
| Soyabean meal | 30.88 |
| Meat-bone meal | 2.80 |
| Fish meal | 9.58 |
| Dicalcium phosphate ^a | 0.96 |
| DL-methionine | 0.15 |
| Salt | 0.12 |
| Vitamin premix ^b | 0.25 |
| Mineral premix ^c | 0.25 |
| Lysine | 0.17 |
| Choline-Cl | 0.22 |
| Total | 100 |
| Calculated analysis | |
| Crude protein | 23.61 |
| ME (MJ/kg) ^d | 12.84 |
| Calcium | 0.89 |
| Phosphorus | 0.67 |
| L-lysine | 1.42 |
| Methionine+cystine | 0.95 |

^a Contains 24% Ca and 17.5% P;

^b Provided per kg of diet: Vitamin A, 12,000 IU; vitamin D₃, 1,500 IU; vitamin E, 50 IU; vitamin K₃, 5 mg; vitamin B₁, 3 mg; vitamin B₂, 3 mg; pyridoxine, 30 mg; vitamin B₁₂, 0.3 mg; pantothenic acid, 12 mg; niacin, 25 mg; D biotin, 0.5 mg; folic acid, 1 mg; choline chloride, 400 mg; apo caretenoic acid ester, 2.5 mg.

^c Provided per kg of diet: iron, 80 mg; zinc, 40 mg; manganese, 60 mg; iodine, 0.8 mg; copper, 8 mg; selenium, 0.2 mg; cobalt, 0.4 mg.

^d ME (MJ/kg): 1 cal = 4.184 J.

Analysis of intestinal pH

At the end of the experimental period, eight birds from each treatment group were selected and sacrificed to evaluate the pH values. The intestine was collected and their pH determined immediately with an electronic pH meter.

Blood biochemical analysis

At the end of the experiment, blood samples were collected from 10 birds per group via the retroorbital venous plexus for hematological and biochemical study. Within one hour of collection the serum was separated. The serum was then analysed for alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity, using the SNA-12 clinical method (Anonymous, 1974). Serum total triglyceride, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and biochemical parameters (total protein, uric acid and glucose) were measured. All of the tests mentioned above were analysed by the fully automated Cobas-Integra 800 instrument (Roche). Internal quality control was achieved by including control samples of known value in at least two different levels of concentration. The external quality assessment programme held by UK-NEQAS covers all the tests.

Samples and chemical analysis of Turkish *Populus spp.* propolis

Propolis sample was collected from Kayseri city in Turkey (North-West Anatolia) in September 2003. Hand-collected propolis samples were kept desiccated in the dark until their processing. Thirty g propolis samples were extracted for a week with 100 ml of 70% ethanol, at room temperature to obtain the extract. After filtration, the extracts were evaporated to dry up under vacuum at 50°C. One mg of dry extract was reacted with 50 µl pyridine+100 µl bis-trimethylsilyl) trifluoroacetamide (BSTFA) including 1% trimethylchlorosilane (TMCS) in a sealed glass tube for 30 min at 100°C to prepare samples for gas chromatography. Sample volumes of 1 µl were injected and analyzed by GC-MS. Gas chromatography-mass spectrometry was carried out on an Agilent GC 6890 gas chromatograph coupled to an Agilent MSD 5973 mass detector under electron impact ionization. The chromatographic column for the analysis was Zebron (ZB-1) methyl polysiloxane column (30 ml×0.25 mm10×0.25 µm). The carrier gas used was helium at a flow rate of 10 ml/min. Propolis sample was analyzed with the column held initially at 100°C for 5 min and then increased to 150°C and then kept at 150°C for 2 min. Finally, temperature was increased to 280°C with a 2°C/min heating ramp and the temperature was kept 280°C for 60 min. Samples were injected in split mode at 250°C. Peaks were identified by computer searches in commercial reference libraries.

Antibacterial analysis Turkish *Populus spp.* propolis

The antimicrobial screening was performed using Mueller Hinton agar (MHA, Oxoid) and MHA with 5% defibrinated sheep blood for bacteria and RPMI-1640 Medium (Sigma) for yeast. Gram positive, Gram negative bacteria and yeast like fungi were used for antimicrobial activity studies. *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 90028) was used as a control. Nineteen bacteria strains and nine fungi strains, isolated from clinical specimens of patients treated at the University Hospital of the School of Medicine of Erciyes, Kayseri-Turkey, were used as follows: *Staphylococcus aureus* (n = 5), *Streptococcus pneumoniae* (n = 4), *Escherichia coli* (n = 6), *Enterococcus faecalis* (n = 4) and *Candida albicans* (n = 9) with standard strains for antimicrobial test. All microorganisms were provided by Department of Microbiology, Faculty of Medicine, Erciyes University. Antimicrobial activity of propolis sample was investigated by the agar dilution method, following the National Committee of Clinical Laboratory Standard guidelines. Bacterial strains were grown in Mueller Hinton Agar (Oxoid) at 37°C/24 h and yeasts in RPMI medium at 25°C/48 h. The turbidity of the suspensions was adjusted to the Mac Farland 0.5 turbidity

standards. Each antimicrobial test also included plates containing the culture medium plus ethanol, in order to obtain a control of the solvent antimicrobial effect. Propolis extracts were added in arithmetical progression. The concentration of propolis in the media was expressed in µg per ml. After the inoculation procedures, plates were incubated at 37°C/24 h (yeasts at 25°C/48 h) and MIC and points were read as the lowest concentration of propolis that resulted in no visible growth on the surface of the culture medium.

Statistical analysis

Data were analysed using the General Linear models (GLM) procedure of SPSS 10.0 (1999) using the following model, a completely randomized design (CRD); $\hat{Y}_i = \mu + \alpha_i + e_{ij}$; Here, \hat{Y}_i : observation value; μ : means of population; α_i : the effect of treatments: control, propolis levels and Flavomycin, respectively, e_{ij} : residual error. Means were separated by using Duncan multiple comparison. Results from feeding treatment diets 1 through 4 were analyzed as an orthogonal polynomial. Linear, quadratic and cubic effects were determined by orthogonal polynomial contrasts.

RESULTS

The chemical components of the propolis used in this study as identified by GC/MS are presented Table 2.

The effect of dietary supplementation of propolis and falavomycin on the growth performance of the quails is presented in Table 3, 4 and 5. Diet supplementation with propolis increased body weight gain and feed consumption ($p < 0.01$) and also improved feed efficiency ($p < 0.01$) during the last four weeks (from 2 to 5 weeks) in the experiment. Birds fed diets containing flavomycin tended to be more efficient than control and propolis groups during in the growing period. For feed consumption and feed efficiency, the effect was both quadratic and cubic, but for body weight gain, linear at the end of study.

The measures for carcass yield and weight, intestinal weight, pH and length and other internal organ weights such as abdominal fat, liver, gizzard and proventriculus are given Table 6. Carcass weight was significantly ($p < 0.01$) higher in quail fed with propolis and flavomycin. In addition, at the end of the study, the group receiving 0.5, 1 and 1.5 g/kg propolis in the diets showed lower liver weights than control and flavomycin groups. However, no differences were noted in gizzard, proventriculus and intestinal weights or intestinal length and intestinal pH.

Enzyme activities and other variables in serum of quail birds given propolis and flavomycin are presented in Table 7. There were significant ($p < 0.05$) decrease in serum ALT activities of birds given 1 g/kg diet propolis compared with flavomycin group. Serum glucose level was significantly ($p < 0.05$) increased in flavomycin group compared with

control and propolis groups. The level of 0.5 or 1 g/kg propolis supplementation increased serum HDL and decreased LDL levels compared with other treatments. However, there were no significant effects among the treatments ($p>0.05$). No differences were observed for serum ALP and AST or for concentration of total protein, uric acid, cholesterol and triglyceride.

DISCUSSION

The results showed that there was a higher growth performance in quails when propolis was included in the diets. The addition of propolis at 0.5, 1 or 1.5 g/kg in the diet significantly increased growth parameters of quail chicks such as body weight gain and feed consumption and improved feed efficiency compared with controls and gave

similar effects to that of flavomycin on body weight gain during a 35 day feeding period. It could be inferred that the antimicrobial activity of the components of the propolis extract, resulted in better intestinal health and improved digestion and absorption. In addition to its antimicrobial activity (Kujumgiev et al., 1999), propolis possesses biological activities such as that of antioxidants (Orhan et al., 1999). This is also confirmed by the chemical analysis of the propolis (Table 2). Our results agree with the findings of Biavatti et al. (2003), who reported that *Alternanthera brasiliana* and propolis extracts increased body weight gain on the 7th day of broilers. Similarly, an improved growth performance in broiler chickens were reported by Alcicek et al. (2003) who used an 48 mg/kg feed essential oil combination (EOC) and Urbanczyk et al. (2002) who investigated a herb mixture (*thymus vulgaris*, *origanum*

Table 2. Chemical composition of propolis (%of total ion current, GC-MS)*

| Retention time (min) | Compounds | % TIC | Retention time (min) | Compounds | % TIC |
|----------------------|-------------------------------|-------|----------------------|-----------------------------------------|--------|
| | Fatty acids | 25.3 | | Esters | 5.7 |
| 46.9 | Octadecanoic acid | | 34.8 | 4,3 acetyloxycaffeate | |
| 14.6 | 9,12,15 Octadecatrienoic acid | | 11.8 | 2-propenoic acid methyl ester | |
| 21.0 | Tetradecanoic acid | | 36.2 | Caffeic acid methyl ester | |
| 51.2 | Undecanoic acid | | | Ketons | 16.8 |
| 30.9 | Oleic acid | | 19.6 | Cyclohexanone | |
| 34.4 | Arachidonic acid | | 21.5 | 3-methyl,antitriecylo undec-3-en 10-one | |
| | Aromatic acids | 6.3 | | Others | 26.6 |
| 5.9 | Benzoic acid | | 51.2 | Cyclohexane | |
| 29.2 | Caffeic acid | | 50.3 | Cyclopentene | |
| 31.2 | Palmitoleic acid | | 42.2 | 5-n-propyl-1,3 dihydroxybenzene | |
| | Alcohol and terpens | 2.02 | | Flavonoids | 17.348 |
| 45.2 | Pentitol | | | Chrysin | |
| 21.0 | Ribitol | | | Pinobanksin | |
| 38.8 | Vanilethanediol | | | | |
| 14.7 | Bicyclohept-3-en-2-ol | | | | |

* The ion current generated depends on the characteristics of the compound concerned and it is not a true quantitation

| Species (no. of strains tested) | MIC ranges (mcg/ml) | MIC ₅₀ (mcg/ml) | MIC ₉₀ (mcg/ml) | Mean inhibition zone Diameter+SE (cm) |
|---------------------------------|---------------------|----------------------------|----------------------------|---------------------------------------|
| <i>S. aureus</i> (n = 5) | <0.1-0.2 | <0.1 | 0.2 | 13.8±1.3 |
| <i>E. coli</i> (n = 6) | 3.5-14.0 | 3.5 | 14.0 | 9.0±1.0 |
| <i>S. pneumoniae</i> (n = 4) | 0.2-3.5 | 0.4 | 0.4 | 11.2±0.8 |
| <i>E. faecalis</i> (n = 4) | 1.75-3.5 | 3.5 | 3.5 | 10.2±0.6 |
| <i>C. albicans</i> (n = 9) | 3.75 | 3.75 | 3.75 | 12.2±0.63 |

Table 3. Effects of dietary propolis and flavomycin on body weight of quails (0-35 d)

| Days | Treatments | | | | SEM | P | Effects | | |
|-------|--------------------|--------------------|--------------------|--------------------|------|----|-----------------------|----|----|
| | Control | Propolis (g/kg) | | | | | Flavomycin (10 mg/kg) | L | Q |
| | | 0.5 | 1 | 1.5 | | | | | |
| 1-7 | 28.1 ^a | 29.4 ^{ab} | 31.1 ^{bc} | 32.2 ^c | 0.38 | ** | ** | ns | ns |
| 8-14 | 48.9 ^a | 67.5 ^c | 61.3 ^b | 63.0 ^b | 0.84 | ** | ** | ** | ** |
| 15-21 | 94.1 ^a | 113.4 ^b | 107.8 ^b | 108.8 ^b | 1.11 | ** | ** | * | * |
| 22-28 | 140.3 ^a | 154.5 ^b | 152.7 ^b | 154.9 ^b | 1.37 | ** | ** | ns | ns |
| 29-35 | 180.1 ^a | 191.7 ^b | 193.4 ^b | 195.5 ^b | 1.63 | ** | ** | ns | ns |

SEM: Pooled standard error of the mean, L: Linear, Q: Quadratic, C: Cubic effects.

^{a, b, c} Means within row with different superscripts differ significantly. * $p<0.05$, ** $p<0.01$.

Table 4. Effects of dietary propolis and flavomycin on feed consumption of quails (0-35 d)

| Days | Treatments | | | | | SEM | P | Effects | | |
|-------|---------------------|--------------------|--------------------|--------------------|-----------------------|------|----|---------|----|----|
| | Control | Propolis (g/kg) | | | Flavomycin (10 mg/kg) | | | L | Q | C |
| | | 0.5 | 1 | 1.5 | | | | | | |
| 1-7 | 50.7 ^d | 49.7 ^b | 50.0 ^c | 48.7 ^a | 50.0 ^c | 0.09 | ** | ** | ** | ** |
| 8-14 | 135.3 ^c | 135.6 ^d | 130.0 ^b | 120.0 ^a | 136.3 ^e | 0.88 | ** | ** | ** | ns |
| 15-21 | 281.1 ^b | 277.6 ^b | 265.4 ^a | 267.3 ^a | 285.0 ^b | 1.59 | ** | ** | ns | * |
| 22-28 | 443.3 ^c | 413.1 ^b | 387.6 ^a | 425.6 ^b | 443.2 ^c | 3.86 | ** | ** | ** | ** |
| 29-35 | 619.8 ^{bc} | 593.8 ^b | 545.1 ^a | 631.3 ^c | 691.9 ^d | 7.36 | ** | ns | ** | ** |

SEM: Pooled standard error of the mean, L: Linear, Q: Quadratic, C: Cubic effects.

^{a, b, c, d, e} Means within row with different superscripts differ significantly, * p<0.05, ** p<0.01.**Table 5.** Effects of dietary propolis and flavomycin on feed efficiency of quails (0-35 d)

| Days | Treatments | | | | | SEM | P | Effects | | |
|-------|-------------------|--------------------|--------------------|--------------------|-----------------------|------|----|---------|----|----|
| | Control | Propolis (g/kg) | | | Flavomycin (10 mg/kg) | | | L | Q | C |
| | | 0.5 | 1 | 1.5 | | | | | | |
| 1-7 | 1.83 ^c | 1.70 ^{bc} | 1.62 ^{ab} | 1.52 ^a | 1.59 ^{ab} | 0.03 | ** | ** | ns | ns |
| 8-14 | 2.57 ^c | 2.06 ^b | 2.11 ^b | 1.91 ^a | 2.13 ^b | 0.04 | ** | ** | * | ** |
| 15-21 | 2.84 ^c | 2.44 ^a | 2.46 ^a | 2.46 ^a | 2.61 ^b | 0.03 | ** | ** | ** | ns |
| 22-28 | 3.10 ^c | 2.68 ^{ab} | 2.54 ^a | 2.75 ^b | 2.80 ^b | 0.03 | ** | ** | ** | ns |
| 29-35 | 3.46 ^d | 3.10 ^b | 2.82 ^a | 3.23 ^{bc} | 3.37 ^{cd} | 0.04 | ** | ** | ** | ** |

SEM: Pooled standard error of the mean L: Linear, Q: Quadratic, C: Cubic effects.

^{a, b, c, d} Means within row with different superscripts differ significantly, * p<0.05, ** p<0.01.**Table 6.** Effects of dietary propolis and flavomycin on carcass characteristics and internal organ properties in quails

| Measurement | Treatments | | | | | SEM | P |
|--------------------------------|--------------------|---------------------|---------------------|---------------------|-----------------------|------|----|
| | Control | Propolis (g/kg) | | | Flavomycin (10 mg/kg) | | |
| | | 0.5 | 1 | 1.5 | | | |
| Carcass weight (CW, g/bird) | 137.3 ^a | 142.3 ^{ab} | 147.6 ^{bc} | 151.6 ^{cd} | 157.7 ^d | 1.58 | ** |
| Carcass yield (%) | 75.6 | 74.1 | 76.0 | 75.9 | 76.8 | 0.44 | ns |
| Abdominal fat weight (g/bird) | 0.8 | 1.2 | 1.4 | 1.4 | 0.86 | 0.10 | ns |
| Liver weight (g/bird) | 3.4 | 3.1 | 3.1 | 3.1 | 3.6 | 0.07 | ns |
| Gizzard weight (g/bird) | 3.7 | 3.8 | 3.8 | 3.6 | 3.6 | 0.08 | ns |
| Intestinal weight (g/bird) | 6.5 | 6.4 | 6.3 | 6.2 | 5.8 | 0.15 | ns |
| Intestinal length (cm/bird) | 62.5 | 62.4 | 58.3 | 62.1 | 60.0 | 0.87 | ns |
| Proventriculus weight (g/bird) | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.01 | ns |
| Intestinal pH | 6.3 | 6.2 | 6.3 | 6.2 | 6.3 | 0.02 | ns |

SEM: Pooled standard error of the mean.

^{a, b, c, d} Means within row with different superscripts differ significantly, * p<0.05, ** p<0.01.

mairana, *coriandrum sativum* and *taraxacum vulgare*) and Demir et al. (2003), thyme and garlic powder, Kucukersan et al. (2002) stabilized rumen extract (SRE) in the diet. However, more experiments are needed to explain whether propolis can affect antimicrobials or antioxidants in poultry diets. In contrast to our results, Botsoglou et al. (2004) found that the addition of essential oils to the broiler diet had no beneficial effect on broiler performance. In another trial, De-Freitas et al. (2001) reported that the supplementation of garlic extract to the broiler diet had no suitable as substitute growth promoters.

The propolis inclusion had no effect on the relative weight and length of the intestine and the relative weight of the gizzard, proventriculus, liver and intestinal pH. These results agree with the finding of Siriken et al. (2003) who reported that a mixture of probiotic (Protexin and Biosacc) added to the diet did not change the intestinal pH of Japanese quails. Similarly, De-Freitas et al. (2001) found

that feed treated with garlic fed to chickens did not affect their intestine weights.

The biochemical changes and alterations in enzyme activities induced a stress on liver function (Abdel-Wahhab et al., 1999). In the present study, there was an increase in AST and ALT activities of birds fed with flavomycin compared to the control. The supplementation of propolis in diet caused a reduction in these enzyme activities compared to flavomycin group. Based on this result, we may argue that propolis may have hepatoprotective effects or play a role in the prevention of liver injury. No significant differences were seen in serum total protein, uric acid total cholesterol and triglyceride between treatment groups. However, quails fed 0.5 and 1 g/kg propolis diets tended to increase serum HDL and decrease LDL levels compared to the control and other treatment groups. These results agree with the findings of Kolonkaya et al. (2002) who studied the effects of adding the Turkish propolis to rat diet

Table 7. Effects of dietary propolis and flavomycin on serum biochemical variables in quails

| Variables | Treatments | | | | | SEM | P |
|----------------------|---------------------|---------------------|---------------------|---------------------|--------------------------|------|----|
| | Control | Propolis (g/kg) | | | Flavomycin (10 mg/kg) | | |
| | | 0.5 | 1 | 1.5 | | | |
| ALP (U/L) | 1,217.4 | 1,182.0 | 1,015.8 | 1,076.2 | 996.6 | 33.9 | ns |
| AST (U/L) | 209.4 ^a | 267.8 ^{ab} | 221.0 ^{ab} | 248.9 ^{ab} | 299.2 ^b | 11.6 | * |
| ALT (U/L) | 3.8 ^a | 4.4 ^{ab} | 3.9 ^a | 5.1 ^{ab} | 5.6 ^b | 0.23 | * |
| Total protein (g/dL) | 2.1 | 2.1 | 2.1 | 2.2 | 2.2 | 0.07 | ns |
| Uric acid (mg/dL) | 3.1 | 3.5 | 2.7 | 3.6 | 3.8 | 0.33 | ns |
| Triglyceride (mg/dL) | 63.4 | 63.0 | 58.4 | 69.6 | 63.6 | 2.81 | ns |
| Cholesterol (mg/dL) | 69.6 | 68.8 | 69.6 | 69.0 | 76.2 | 2.28 | ns |
| HDL (mg/dL) | 142.7 | 165.7 | 146.6 | 164.3 | 184.6 | 8.63 | ns |
| LDL (mg/dL) | 38.5 | 26.5 | 22.3 | 23.6 | 29.2 | 2.66 | ns |
| Glucose (mg/dL) | 324.7 ^{ab} | 323.8 ^{ab} | 333.8 ^{ab} | 294.8 ^a | 344.9 ^b | 6.90 | * |

SEM: Pooled standard error of the mean.

^{a, b, c, d} Means within row with different superscripts differ significantly, * p<0.05, ** p<0.01.

observed similar effects to those for the present study. Similar results in improvement of serum HDL and LDL have been obtained by the addition of probiotics (*Lactobacillus* or *L. cultures*) in the diet of broiler chickens (Mohan et al., 1996; Pietras, 2001; Kalavathy et al., 2003). Our study showed that the propolis positively affected the HDL and LDL levels of the quail chicks. But, there is clear need to clarify the constituents of propolis in order to evaluate its biological activity.

In conclusion, this study demonstrated that propolis supplementation particularly at 1 g/kg feed, increased the growth performance and improved the serum lipid variables such as HDL and LDL of quails. In addition, propolis and flavomycin during the growth period showed similar effects on growth performance. Therefore, it may serve as a natural substitute for antibiotics in poultry diets.

REFERENCES

- Abdel-Wahhab, M. A., S. A. Nada and F. A. Khalik. 1999. Physiological and toxicological responses in rats fed aflatoxin-contaminated diet with or without sorbent materials. *Anim. Feed Sci. Technol.* 97:209-219.
- Alcicek, A., M. Bozkurt and M. Cabuk. 2003. The effect of an essential oil combination derived from selected herbs growing wild in Turkey on broiler performance. *S. Afr. J. Anim. Sci.* 33:89-94.
- Amaros, M., C. M. O. Simoes, L. Girre, F. Sauvager and M. Cormier. 1994. Comparison of the anti Herpes simplex virus activities of propolis and 3-methyl-butyl -2-enyl caffeate. *J. Nat. Prod.* 57:644-647.
- Anonymous. 1974. Technical publication No. UA4-0160-00, Technion Instr. Corp., Terrytown, 1974. pp. 127-129.
- Banskota, A. H., Y. Tezuka, K. Midorikawa, K. Matsushige and S. Kadota. 2000. Two novel cytotoxic benzofuran derivatives from Brazilian propolis. *J. Nat. Prod.* 63(9):1277-1279.
- Biavatti, M. W., M. H. Bellaver, L. Volpato, C. Costa and C. Bellaver. 2003. Preliminary studies of alternative feed additives for broilers: *Alternanthera brasiliana* extract, propolis extract and linseed oil. *Rev. Bras. Cienc. Avic.* 5(2):147-151.
- Botsoglou, N. A., E. Christaki, P. Florou-Paneri, I. Giannenas, G. Papageorgiou and A. B. Spais. 2004. The effect of mixture of herbal essential oils or α -tocopheryl acetate on performance parameters and oxidation of body lipid in broilers. *S. Afr. J. Anim. Sci.* 34:52-61.
- Demir, E., S. Sarica, M. A. Ozcan and M. Suicmez. 2003. The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets. *Br. Poult. Sci.* 44:44-45.
- De-Freitas, R., J. B. Fonseca, R. D. R. N. Soares, H. S. Rostagno and P. R. Soares. 2001. Utilization of garlic (*Allium sativum* L.) as growth promoter of broilers. *Rev. Bras. Zootecnia.* 30(3):761-765.
- Deschner, E. E., J. Ruperto, G. Wonk and H. L. Newmark. 1991. Quercetin and rutin as inhibitors of azoxymethanol induced colonic neoplasia. *Carcinogenesis* 12:771-775.
- Dimov, V., N. Ivanovska, N. Manolova, V. Bankova, N. Nikolov and S. Popov. 1991. Immunomodulatory action of propolis. Influence on anti-infectious protection and macrophage function. *Apidologie* 22:155-162.
- Ghisalberti, E. L. 1979. Propolis: a review. *Bee world* 60:59-84.
- Kalavathy, R., N. Abdullah, S. Jalaludin and Y. W. Ho. 2003. Effects of *Lactobacillus* cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. *Br. Poult. Sci.* 44(1):139-144.
- Keskin, N., S. Hazir, K. H. C. Baser and M. Kurkcuoglu. 2001. Antibacterial activity and chemical composition of Turkish propolis. *Z. Naturforsch* 56:1112-1115.
- Kolonkaya, D., G. Selmanoglu, K. Sorkun and B. Salih. 2002. Protective effects of Turkish propolis on alcohol-induced serum lipid changes and liver injury in male rats. *Food Chemistry* 78:213-217.
- Kucukersan, K., S. D. Tuncer, Y. Sanli, M. Midilli, E. Goncuoglu, S. Kucukersan and H. Tan. 2002. The effects of dietary stabilized rumen extract (SRE) and virginamycine on performance and carcass yield of broilers. *Revue De Medicine Veterinaire* 153(11):723-726.
- Kujumgiev, A., I. Tsvetkova, Yu. Serkedjieva, V. Bankova, R. Christov and S. Popov. 1999. Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. *J. Ethnopharmacology* 64:235-240.
- Manolovan, M., V. Maximova, G. Gegova, Y. Serkedjieva, S. Uzunov, N. Marekov and V. Bankova. 1985. On the influenza action of fractions from propolis. *Comptes rendus de*

- I'academie de Bulgare de Sciences 38:735-738.
- Matsuno, T., Y. Matsumoto, M. Saito and J. Morikawa. 1997. Isolation and characterization of cytotoxic diterpenoid isomers from Propolis. *Z. Naturforsch (52c)*:702-704.
- Miyataka, H., M. Nishiki, H. Matsumoto, T. Fujimoto, M. Matsuka and T. Satoh. 1997. Evaluation of propolis. I. Evaluation of Brazilian and Chinese propolis by enzymatic and physico-chemical methods. *Biological and Pharmaceutical Bulletin* 20:496-501.
- Mohan, B., R. Kadirvel, A. Natarajan and M. Bhaskaran. 1996. Effect of probiotic supplementation on growth, nitrogen utilisation and serum cholesterol in broilers. *Br. Poult. Sci.* 37(2):395-401.
- National Research Council. 1994. *Nutrient Requirements of Poultry*. 9th rev. Ed. National Academy Press, Washington, DC.
- Orhan, H., S. Marol, I. F. Hepsten and G. Sahin. 1999. Effects of some probable antioxidants on selenite-induced cataract formation and oxidative stress-related parameters in rats. *Toxicology* 139(3):219-232.
- Ota, C., C. Unterkircher, V. Fantinato and M. T. Shimizu. 2001. Antifungal activity of propolis on different species of *Candida*. *Mycoses* 44:375-378.
- Paintz, M. and J. Metzner. 1979. Zur lokalanasthetischen Wirkung von Propolis und einigen Inhaltsstoffen. *Pharmazie* 34:839-841.
- Pietras, M. 2001. The effect of probiotics on selected blood and meat parameters of broiler chickens. *J. Anim. Feed Sci.* 10:297-302.
- Popova, M., S. Silici, O. Kaftanoglu and V. Bankova. 2005. Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. *Phytomedicine* (in press).
- Revington, B. 2002. Feeding poultry in the post-antibiotic era. *Multi-State Poult. Meeting* May 14-16. pp. 1-14.
- Sawaya, A. C. H. F., A. M. Palma, F. M. Caetano, M. C. Marcucci, I. B. Silvacunha, C. E. P. Araujo and M. T. Shimizu. 2002. Comparative study of *in vitro* methods used to analyse the activity of propolis extracts with different compositions against species of *Candida*. *Letters in Applied Microbiology* 35:203-207.
- Sforcin, J. M., J. A. Fernandes, C. Lopes, V. Bankova and S. R. C. Funari. 2000. Seasonal effect on Brazilian propolis antibacterial activity. *J. Ethnopharmacology* 73:243-249.
- Silici, S. and O. Kaftanoglu. 2003. Antimicrobial analysis of propolis samples from different regions of Turkey. *Uludag Bee J.* 3(3):16-18.
- Siriken, B., I. Bayram and A. G. Onol. 2003. Effects of probiotics: alone and in a mixture of Biosacc[®] plus Zinc Bacitracin on the caecal microflora of Japanese quail. *Res. Vet. Sci.* 75(1):9-14.
- SPSS, Inc. 1999. *SPSS for Windows*. Release, 10.0, Copyright SPSS inc., Chicago, USA.
- Sud'ina, G. F., O. K. Mirzoeva and M. A. Pushkareva. 1993. Caffeic acid phenethyl ester as a lipoxygenase inhibitor with antioxidant properties. *FEBS Lett.* 329:21-24.
- Urbanczyk, J., E. Hanczakowska and M. Swiatkiewicz. 2002. Herb mixture as an antibiotic substitute in pig feeding. *Medycyna Weterynaryjna* 58(11):887-889.
- Velikova, M., V. Bankova, K. Sorkun, S. Houcine, I. Tsvetkova and A. Kujumgiev. 2000. Propolis from Mediterranean Region: Chemical composition and antimicrobial activity. *Z. Naturforsch* 55:790-793.
- Verma, A. K., J. A. Johnson, M. N. Gould and M. A. Tanner. 1988. Inhibition of 7,12-dimethylbenz [a] anthracene and N-nitrosomethylurea-induced rat mammary cancer by dietary flavonol quercetin. *Cancer Res.* 48:5754-5758.