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Effect of Propolis Supplementation on Serum Calcium, Phosphorus and Proteins Concentrations in Heat Stressed Broilers

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Abstract

The objective of this study was to investigate the effects of Chinese propolis dietary supplementations on serum total calcium, phosphorus, albumin, globulin and total protein of broilers exposed to chronic heat stress. Whereas heat stressed broilers fed with basal diets (controls) and supplemented with Propolis (250 mg/kg/day) or with Propolis (500 mg/kg/day) or with Propolis (750 mg/kg/day) were exposed to 38.0±1.4°C. Serum biochemical markers, serum calcium (Ca), phosphorus (P), albumin, globulin and total protein were assessed at the end of the experimental period. Serum concentrations of total proteins, albumin, globulins, calcium, phosphorus, were not significantly altered compared to the controls. Dietary supplementations of low dose of propolis not significantly increase the serum total protein concentration, total globulin, Ca: P ratio and serum calcium concentration, while, reduced the serum phosphorus level and the albumin: globulin ratio. But, the higher doses had a conversely effect.

Keywords: Blood indicators; Broilers; Heat stress; Propolis

Introduction

In many countries of the world, particularly in the hot and humid tropics, broiler chickens are often maintained at environmental temperatures above the zone of thermoneutrality. In practice, this has a negative influence on performance. It is well documented that high environmental temperatures depress food intake and body weight and also cause deterioration in the food conversion ratio (McNaughton and Reece 1984; Donkoh 1989).

Recently, it has been found that the natural feed additives like herbs and edible plants have some properties as growth enhancers, instead of using synthetic drugs which may have adverse effects on human health (Fluck *et al*., 1976). Consequently, there is an increase demand for using natural feed additives to overcome the adverse effect of heat stress on broiler performance in tropical and subtropical environments.

It has been reported that the natural feed additives like propolis (bee glue) have some properties as growth enhancers to improve birds productive performance under normal or stress conditions (Biavatti *et al*., 2003; Denli *et al*., 2004; Shalmany and Shivazad, 2006; Tatli Seven *et al*., 2008; Galal *et al*., 2008; Hassan and Abdulla, 2011; Daneshmand *et al*., 2012).

The composition and properties of Propolis have been fairly investigated and considered to be a good source of antioxidant, antimicrobial, immunomodulatory and anti-inflammatory substances (Dobrowolski *et al*., 1991; Bankova, 2005). However, its effectiveness are believed to be due to a combination of aromatic acids, diterpenic acids, flavonoids and phenolic compounds which are responsible for the biological activities of propolis samples (Haile *et al*., 2012).

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Many trials have been conducted to investigate the possibility of introducing Propolis as natural feed additive to broiler diets for better performance. There is a general agreement that Propolis has no adverse effects on performance of broiler and could be supplemented to overcome the deleterious effects of hot climatic conditions (Seven *et al*., 2008; Tatlı Seven, 2008). Also, supplementing broiler diets with Propolis improve growth performance, biochemical and hematological responses and mortality rate (Omar *et al*., 2004; Zhi-jiang *et al*., 2004; Shalmany and Shivazad 2006; Hassan and Abdulla, 2011), and has a stimulatory effect on the immune system (Taheri *et al*., 2005; Shihab and Ali, 2012), however, it's full potential as an anti-stress factor has not been exploited.

Studies with propolis are usually used to enhance growth by making use of antimicrobial and anti-oxidant properties. However, data about the participation of propolis in broiler diet as anti-stress are scarce. The purpose of this study was to determine the adaption mechanisms and the responses to heat stress in broilers during entire feeding period on serum proteins and calcium and phosphorus status and how propolis supplementation influence these mechanisms as anti-stress factors.

Materials and methods

Propolis

Commercial propolis produced by Dalian Tianshan Industrial Co., Ltd. Changjiang Road, Dalian, Liaoning, China.

Experiment design and treatments

The study was conducted at hot climate conditions (July and August 2010) in Animal Hygiene Department, Faculty of Veterinary Medicine, Assiut University, Egypt. A total of sixty four (one day-old) Ross chicks provided by Elwady chicks Company (Assiut, Egypt), were used in the study. The birds were randomly assigned, according to their initial body weights, to four treatment groups, 16 birds each. All pens were bedded with a wood-shavings litter and equipped with feeders and waterier in environmental chambers.

During the experimental period (from 15 to 42 days of age), the birds were kept under 38.0±1.4°C and 49.0±2.0% RH. The heat source was provided by electrical heaters. Chicks were randomly divided into one control and three treatment groups. Maize and soybean meal-based diets were formulated according to the requirements proposed by the NRC (1994). Diets were formulated as starter and finishing diets (Table1). The experimental groups were as follows; group I (control) were fed with a basal diet, group II was fed with basal diet supplemented with 250 mg propolis /kg diet, group III was fed with basal diet supplemented with 500mg propolis /kg diet. Group IV was fed with basal diet supplemented with 750 mg propolis /kg diet. Small amounts of the basal diet were first mixed with the respective a mounts of propolis as a small batch, and then with a larger amount of the basal diet until the total amount of the respective diets were homogeneously mixed. The birds were fed with starter diet until 21 days of age, followed by finishing diet until 42 days of age.

Table 1. Composition of the experimental diets (g/kg)

Ingredients	Starter	Grower
Corn	50.5	60.05
Fish meal	3.5	3
SBM	36.75	29
Sunflower oil	6	4.7
Dicalciumphoshate	1.5	1.5
Ground lime stone	1	1
Salt	0.3	0.3
Lysine	0.1	0.1
Methionine	0.1	0.1
Premix	0.25	0.25
Calculated nutrient content		
Ingredients	Starter	Grower
ME (Kcal/kg)	3202.465	3208.048
CP (%)	22.977	20.0363

Lighting

Continuous lighting program (23 hours lightning: 1 hour darkness) was used. 60 watt bulb was suspended 2.20 m at head height of the birds. Light intensity at the level of the birds was approximately 2.66 lux/m2/second (Measured by the digital lux meter at the bird head level).

Vaccination Program

Lives Newcastle Disease Virus (NDV)-vaccine was administered in drinking water at 6, 14, 21 and 32 days of age, while the live Infectious Bursal Disease Virus vaccine was given in drinking water at 10, 18 and 25 days of age.

Serum collection and blood analysis

At 42 days old, 6 birds were randomly taken from each treatment, slaughtered. During the bird's exsanguinations 3 cm^3 of blood from each bird was collected in test tube without anti-coagulant to determine the chemical blood parameters and hormones. The tubes were kept at the room temperature for 30 minutes then stored at a refrigerator for 60-90 minutes and then centrifuged at 3000 r.p.m for 10 minutes and the separated serum was transferred to another Epindoorf`s tube using micropipette. The sera were kept at –20ºC, until analysis using commercial kit according to the procedure outlined by manufacturer.

Total serum proteins, albumin, inorganic phosphorus (P) and calcium (Ca) were estimated using commercial kits supplied by Spectrum Diagnostics (Egyptian Company for Biotechnology, Cairo, Egypt).and by means of Digital- VIS/ultraviolet spectrophotometer (Cecil instruments, Cambridge, England, Series NO. 52.232) in the research laboratory at the Department of Animal Medicine, Assuit University, Egypt.

Statistical analysis

The results were expressed as the mean ± SEM. All data were analyzed using one way analysis of variances (ANOVA) followed by Duncan Test using SPSS11.0 statistical software (SPSS, Inc., Chicago, IL, 2001).

Results

The results of this study were presented in Tables 2 and 3. There were no significant differences between the groups of birds treated with propolis and the control group regarding these serum parameters.

The results of serum calcium and phosphorus levels were presented in Table 2. In this study, the addition of 250 mg of propolis had a non-significantly (P>0.05) positive effect on serum calcium level in respect to control group. However, Calcium level was non-significantly (P >0.05) lower in the 500 and 750 mg propolis treated groups in comparison to the control.

Total phosphorus concentration was not significantly lower in 250mg propolis treated group, while it was not significantly increasedin 500 and 750 mg/kg-1propolis as compared to the heat stress untreated group.

The results of the Ca:P ratio showed that addition of 250mg propolis not significantly (p>0.05) increased the Ca:P, while the addition of 500 or 750 mg of propolis not significantly (p>0.05) reduced the Ca:P in comparison to the control group.

The results of effect of diets containing different

Table 2. Effect of diets containing different levels of propolis on serum Ca, P levels in Ross 308 broilers reared under heat stress.

Treatment	Serum calcium concentration (mg/dl)	Serum phosphorus concentration (mg/dl)	Ca/P ratio
Control	7.85±0.56	3.84±0.37	1.99±0.16
250 mg propolis	8.45±1.22	3.32±0.15	2.27±0.32
500 mg propolis	7.26±0.43	3.98±0.09	1.92±0.02
750 mg propolis	7.58±0.40	4.23±0.45	1.77±0.16

Table 3. Effect of diets containing different levels of propolis on serum albumin, globulin and Total protein levels in Ross 308 broilers reared under heat stress.

Treatment	Serum Total protein concentration (g/dl)	Serum Total albumin concentration (g/dl)	Serum Total globulin concentration (g/dl)	Albumin : globulin ratio
Control	$3.85±0.06^{ab}$	1.85±0.038	$1.99±0.05^{ab}$	$0.93±0.03^{ab}$
250 mg propolis	$4.22±0.17^{a}$	1.86±0.086	$2.35±0.16^{a}$	$0.81±0.08^{b}$
500 mg propolis	$3.58±0.18^{b}$	1.89±0.087	$1.68±0.10^{b}$	$1.13±0.05^{ab}$
750 mg propolis	$3.83±0.16b^{b}$	2.09±0.087	$1.73±0.16^{b}$	$1.26±0.13^{a}$

Means with different letters in the same column differ significantly (p<0.05) levels of propolis on serum albumin, globulin and Total protein levels presented in Table 3, shown no significant differences between the groups of birds treated with propolis and the control group. However, there was a significant difference ($p < 0.05$) between 250 mg propolis treated group and 500 and 750 mg propolis treated groups. The addition of low doses of propolis not significantly increase the serum total protein concentration, total globulin concentration and reduced the albumin: globulin ratio. But, the highest doses had the conversely affect.

Discussion

Heat stress is an important stressor resulting in the reduced welfare of birds. Administration of a supplement having anti-stress feature is one of the methods used to prevent the negative effects caused by metabolic stress (Lin *et al*., 2006; Attia *et al*., 2011). Unfortunately, very little published research exists about the effect of propolis supplementation on broiler adaption to the heat stress.

Mineral concentrations might be decreased during stress because of the increased metabolism rate induced by stress hormones. Calcium and phosphorus were slightly, but insignificantly decreased in quail or broilers exposed to heat stress (Seyrek *et al*.2004; Imik *et al*., 2013). McCormick and Garlich (1982) reported that, total plasma calcium and phosphorus in chicks exposed to high temperature (41°C and 43 °C) at 4 weeks old were significantly decreased in all chicks at each bleeding. Also, the calcium / phosphorus ratio was significantly increased at prostration phase. Allahverdi (2013) suggested that, the decrease in calcium level in blood in heat stressed laying hens was primarily related to panting which reduce the body temperature. Moreover, Rama Rao *et al*. (2002) reported that, heat stress reduced calcium intake as well as the conversion of vitamin D_3 to its metabolically active form, 1,25 $(OH)_2D_3$, which is essential for the absorption and utilization of Ca.

In the present study, the results of serum calcium and phosphorus levels and ca: ph ratios were presented in Table 2. The results indicated that the dietary supplementation of propolis has no significant effect on serum calcium and phosphorus level in broilers reared under heat stress. However, the addition of 250 mg of propolis had a non-significant ($P>0.05$) positive effect on the serum calcium level in respect to control group. On contrast, Calcium level was non-significantly ($P >0.05$) lower in the 500 and 750 mg propolis treated groups in comparison to the control. This could be supported by the finding of Petruška *et al*. (2012) who recorded that addition of propolis450 mg.kg to the feeding mixture for broiler chickens for 42 days caused significant decrease of serum phosphorus. Haro *et al*. (2000) clarified that the addition of Propolis caused an increase of phosphorus in the femur and sternum bones, suggesting that propolis inclusion in the diet increases absorption of phosphorus and from the blood to the bone and thus decreased the level of this element in the blood. Also the non-significant increase in serum calcium level in group supplemented by 250 mg propolis, could be explained according Haro *et al*. (2000) to the increase in the digestibility of calcium, due to the acid derivates, such as benzoic, 4-hydroxy-benzoic, etc., which are found in propolis.

It is already established that the total protein, albumin and globulin concentration decreased significantly when birds exposed to heat stress. This decline in blood protein levels in heat-stressed birds may be due to reduced protein synthesis (Vo *et al*., 1978; Faltas *et al*., 1987; Hamoud *et al*., 1993; Zhou *et al*., 1998). In this study the results shown in Table (3), serum total proteins, albumin (A) and globulin (G) as well as A/G ratios were slightly affected by feeding treated diets compared to the control, but differences failed to be significant. The results of the present study were in agreement with those reported by Tatlı Seven *et al*. (2008), who found that the plasma albumins and total protein levels of broilers exposed to heat stress (at 34°C) were not influenced by dietary supplementation with different ethanol extracts of propolis concentrations (EEP) (0.5, 1, 3 g EEP/kg). Moreover, Abdel-Rahman and Mosaad, (2013) indicated that Adding propolis (2g /kg) to the diet of Muscovy ducks maintained at 33°C was reflected with significant higher contents of its serum total protein, albumin and total globulin. The data were 5.24, 3.21, 2.023 and 7.92, 4.41, 3.51 g/dl for control and propolis group, respectively. They attributed the improvement of serum total protein and its fractions in the group fed propolis may be related to its stimulating effect on the liver exhibiting an anabolic action favoring protein synthesis and also it's preserving effect on the body protein from degeneration.

This study results showed that TP, A and G concentrations of birds supplemented with 250mgkg-1 propolis were slightly increased to the normal ranges of strain. The normal globulin values indicate good immunity status of the heat stressed chicks. The slight increase in globulins may be due to the immuno-stimulant effect of propolis (Diker *et al*., 1998; Çetin *et al*., 2010; Shihab and Ali 2012; Abdel-Rahman and Mosaad, 2013).

Conclusion

This present study was designed to assess the effect of Chinese propolis supplementation on serum Ca, Ph. And protein concentration in commercial broilers (Ross308) exposed to chronic heat stress. In conclusion, results showed that propolis extract in amounts that used in the present study does not affect significantly on these biochemical parameters. However, low doses of propolis had not significantly reduced the effect of heat stress on these indicators. So, we recommended more future investigations, especially by using lower doses as it is required tobetter understand the bird's response to the propolis and its different constituents.

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