



Effects of Propolis, Ascorbic Acid and Vitamin E on Thyroid and Corticosterone Hormones in Heat Stressed Broilers

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Abstract

The study was designed to evaluate the effect of oxidative stress during the summer on serum levels of Triiodothyronine (T_3), thyroxine (T_4) and corticosterone hormones in Ross 308 broiler chickens and to compare the efficacy of propolis, ascorbic acid and vitamin E (α -tocopherol acetate) as antioxidants in amelioration of heat stress and normalization of serum T_3 and T_4 and corticosterone levels in broilers. Birds were divided into five groups during the experimental period (15 to 42 days of age), the positive control group (G1) was kept under thermo-neutral condition ($28.0 \pm 4.0^\circ\text{C}$) and fed control diet, while the other four groups were exposed to heat stress ($38.0 \pm 1.4^\circ\text{C}$), control group (G2) chicks were fed control diet without additives, propolis group (G3) chicks were supplemented with 250 mg Chinese ether extracted propolis /kg diet, group (G4) supplemented with 250 mg ascorbic acid/kg diet and group (G5) supplemented with 250 mg of α -tocopherol acetate/kg diet (vitamin E). At the end of day 42, five broilers were randomly chosen from each treatment and slaughtered, and then sera were collected to estimate T_3 and T_4 and corticosterone levels. The results indicated that dietary supplementation of broilers with 250 mg/kg diet propolis, vitamin E or vitamin C can attenuate heat stress induced oxidative damage. These positive effects were evidenced by reduced serum corticosterone levels and improvement in thyroid hormonal levels in comparison to non-supplemented birds reared under heat stress.

Keywords: Propolis; Broiler chickens; Thyroid hormones; Corticosterone

Introduction

Heat stress is a worldwide problem in poultry production and causes enormous economic losses every year. The mechanisms underlying the disadvantageous effect of heat stress involve not only the disturbance in acid base and water balance, but also involve changed endocrine function. Endocrinal changes occur in response to different types of stress in order to cope with the demands of a homeostatic challenge (Miller and O'Callaghan, 2002).

Thyroid hormones are known to be influenced by stress (Miller and O'Callaghan, 2002) and act on multiple metabolic processes affecting the concentration and activity of numerous enzymes, the

metabolism of substrates, vitamins and minerals, and the response of target tissues to several hormones (Yen, 2001). Several researchers reported reduced concentrations of T_3 and T_4 in heat-stressed chickens (Bowen *et al.*, 1984). The inverse relationship between plasma concentration of T_3 and environmental temperature has been also well-known (May *et al.*, 1986; Iqbal *et al.*, 1990).

Environmental stress causes oxidative stress and impairs antioxidants status *in vivo* (Sahin *et al.*, 2001a). Dietary modifications are among the most preferred and practical ways to alleviate the effect of high environmental temperature in poultry (Sahin *et al.*, 2004). Antioxidant supplementation, therefore, has been shown to be beneficial in attenuating the adverse effects of environmental stress (Kafri and Cherry, 1984) and stress induced tissue damage (Sen, 2001; Minka and Ayo, 2007).

Propolis is an adhesive, dark yellow to brown

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colored balsam that smells like resin. It is collected from buds, leaves and similar parts of trees and plants by bees and mixed with wax, sugar and plant exudates collected by bees from certain plant sources. More than 300 constituents have been identified in different propolis samples (Valle, 2000; Banskota *et al.*, 2001; Shalmany and Shivazad, 2006). Propolis usually contains variety of chemical compounds, such as polyphenols (flavonoids, phenolic acids and their esters), terpenoids, steroids and amino acids. The composition of propolis depends on the vegetation at the site of collection (Kumazawa *et al.*, 2003). Propolis has many positive effects like increase in feed intake, body weight increase, flavonoid content, taste improvement, antioxidant and antimicrobial properties. Antioxidative, cytostatic, anti-mutagenic and immunomodulatory properties of propolis are based on its rich, flavonoid, phenolic acid and terpenoid contents (Kimoto *et al.*, 1999; Prytzyk *et al.*, 2003; Wang *et al.*, 2004).

Vitamin C, or L-ascorbic acid, is a water-soluble vitamin widely distributed in plants and animals. It is of major importance in nutrition to maintain a good health status. Generally, ascorbic acid is not regarded as a dietary requirement for poultry because it can be synthesized at a sufficient rate to meet the needs under normal conditions. May and McNaughton (1980) could not demonstrate a positive effect of 0.1% ascorbic acid supplementation on body weight of broiler chickens and did not find any effects on thyroid hormone functions. However, dietary vitamin C has been reported to improve resistance to a variety of stressors including environmental (e.g. heat stress), nutritional and pathological conditions (Agudelo, 1983).

Vitamin E, or α -tocopherol, another antioxidant, has high lipid solubility and is located in plasma and organelle membranes such as mitochondria. Vitamin E is essential in maintaining the integrity of the cardiovascular and other systems. It is considered as the major chain breaking antioxidant that scavenges oxygen free radicals and prevents further peroxidative damage of cell membranes (Bottje and Wideman, 1995; Lorenzoni and Ruiz-Feria, 2006). There are reports that the antioxidant properties of oxidized vitamin E can also be restored by vitamin C. As vitamin C is able to donate an electron to the tocopherol radical generating the reduced antioxidant form of vitamin E, suggests that a major function of vitamin C is to recycle the vi-

tamin E radical (Bottje and Wideman, 1995).

The objective of this study was to evaluate oxidative stress during the summer and to compare the efficacy of propolis, ascorbic acid and vitamin E (α -tocopherol acetate) as antioxidants in amelioration of heat stress and normalization of serum T3 and T4 and corticosterone markers in broilers.

Materials and methods

Birds and experimental design

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 eighty (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 five treatment groups, 16 birds each. All pens were bedded with a wood-shavings litter and equipped with feeders and waterer in environmental chambers.

During the experimental period (15 to 42 days of age), the positive control group (G1) was kept under thermo neutral condition and fed control diet, the average temperature was $28.0 \pm 4.0^\circ\text{C}$ and room humidity (RH) was $55.0 \pm 3.0\%$. While, the other four groups were kept under $38.0 \pm 1.4^\circ\text{C}$ and $49.0 \pm 2.0\%$ RH. The heat source was provided by electrical heaters. Chicks in heat stress treatments were fed control diet (G2) without additives or with 250 mg Chinese ether extracted propolis (EEP)/kg (G3) diet with 250 mg ascorbic acid/kg diet (G4), 250 mg of α -tocopherol acetate/kg diet (vitamin E) (G5).

Feed and Rearing

Feed was administered ad libitum, (Starter ration: age 1-21 days; grower ration: age 22-42 days) were the same for all the five treatments (Table 1), and formulated according to the recommendations of the National Research Council of the US (National Research Council, 1994).

Vaccination Program

Live New Castle Disease Virus (NDV) vaccine was administered in drinking water at 6, 14, 21, and 32 days of age, while the live Infectious Bursal Dis-

ease Virus (IBDV) vaccine was given in drinking water at 10, 18 and 25 days of age.

Table 1. Composition and calculated nutrient content of the experimental diets (%)

Ingredients	Starter	Grower
Corn	50.50	60.05
Fish meal	3.50	3.0
SBM	36.75	29.00
Sunflower oil	6.0	4.7
Dicalcium phosphate	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		
ME (Kcal/kg)	3202.465	3208.048
CP (%)	22.977	20.0363

Sera collection and analysis

At the end of day 42, five birds were randomly chosen from each treatment, slaughtered and then blood samples were collected in centrifuge tubes without anticoagulant. Blood samples were centrifuged at 3000 rpm for 10 min, then serum was collected and stored at -20°C for later analysis.

Serum T_3 , T_4 , and corticosterone concentrations were determined using commercially available Assay Max Corticosterone ELISA kits according to the procedures described by manufacture (Assay pro LLC Saint Charles, Missouri, USA). A commercially available direct ELISA assay (Diametra Srl, Segrate, Italy) was utilized for the total T_3 and total T_4 analysis.

Statistical analysis

The results were expressed as the mean \pm SE. All data were analyzed using one way analysis of variances (ANOVA) followed by Duncan Test using SPSS 11.0 statistical software (SPSS, Inc., Chicago, IL, 2001), www.spss.com.

Results

The results of this study were presented in Figs. 1 to 3. There were no significant differences between the groups of birds treated with propolis, ascorbic acid, vitamin E and the control group regarding these serum parameters.

During the period of heat stress, untreated heat stressed birds showed significant increase in serum corticosterone level when compared with birds

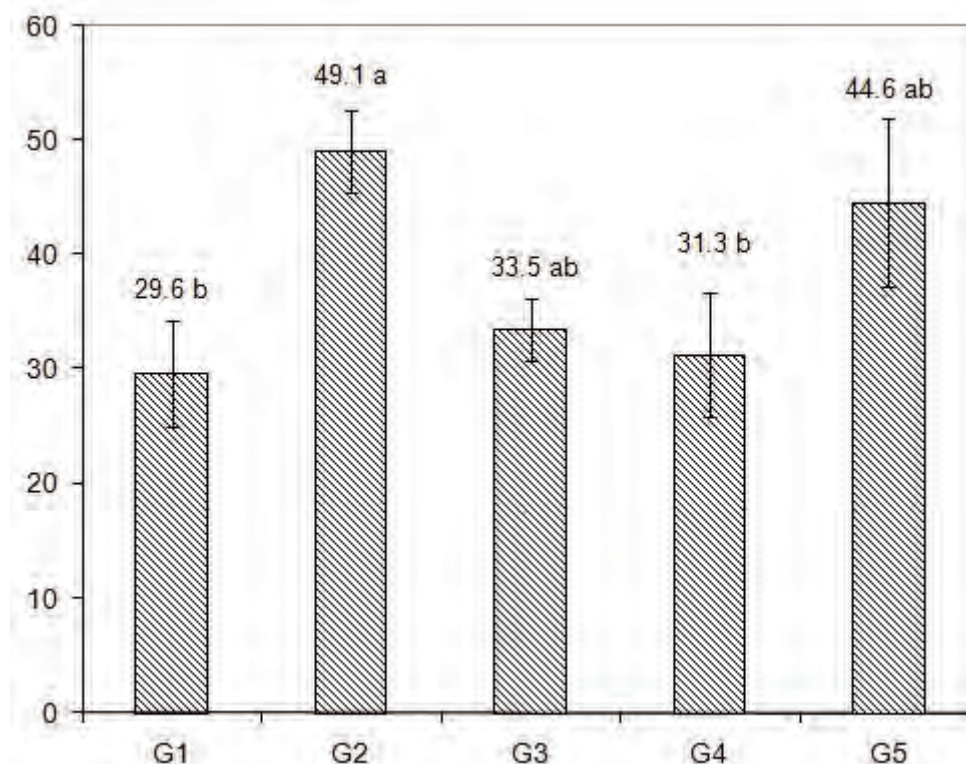


Fig. 1. Effect of treatment with propolis (G3), ascorbic acid (G4), Vit. E (G5) on serum corticosterone (ng/ml) level in Ross broilers reared under heat stress (G2). Values are each mean \pm SE, ANOVA followed Duncan's multiple range test ($P < 0.05$); means having same superscripts does not differ significantly at 5% level.

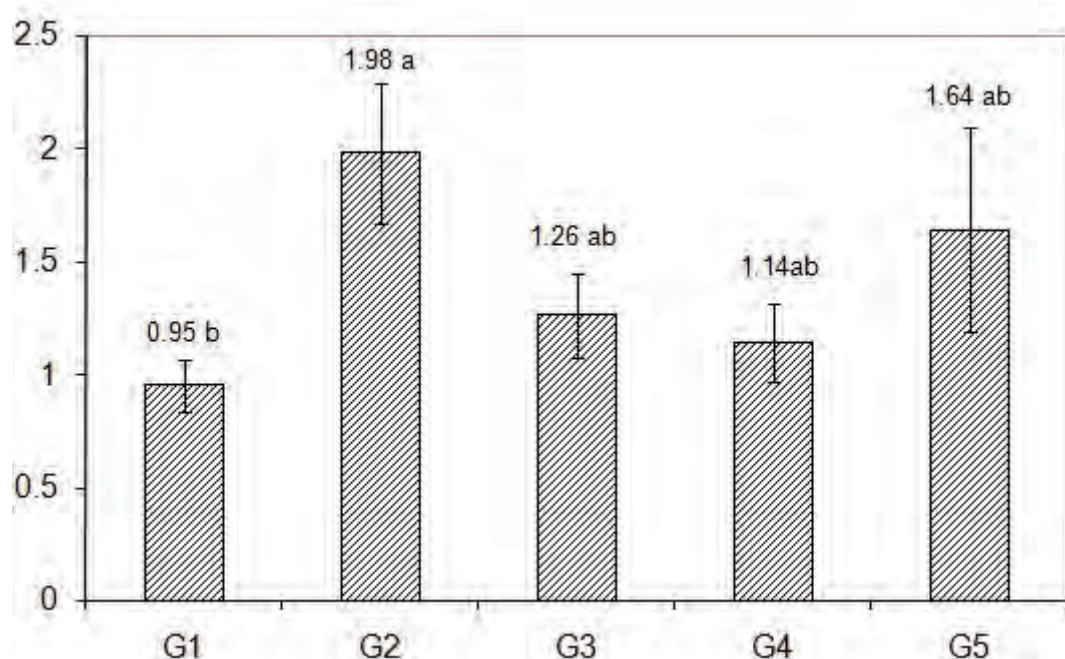


Fig. 2. Effect of treatment with Propolis (G3), ascorbic acid (G4), Vit E (G5) on serum triiodothyronine (ng/ml) level in Ross broilers reared under heat stress. Values are each mean \pm SE, ANOVA followed Duncan's multiple range test ($P < 0.05$); means having same superscripts does not differ significantly at 5% level.

reared under thermo-neutral condition and vitamin C treated groups, while, when compared with propolis and vitamin E treated birds only non-significant differences was recorded (Fig. 1).

In this study, the addition of 250 mg of propolis, ascorbic acid or vitamin E had a positive effect on serum corticosterone level in respect to heat stress group. Total serum corticosterone level was significantly ($P \leq 0.05$) lower in the thermo-neutral treated group (29.60 ± 4.64 ng/ml) and synthetic vitamin C (31.30 ± 5.44 ng/ml), while non-significantly reduced in the propolis (33.50 ± 2.64 ng/ml) followed by vitamin E (44.60 ± 7.33 ng/ml) as it compared to untreated heat stress group (49.10 ± 3.59 ng/ml).

The serum T_3 level at each treatment was summarized in Fig. 2. There were no significant differences in means of the serum T_3 levels between the different treatments. Only significant differences was recorded between untreated heat stressed birds and birds reared under thermo-neutral conditions.

Total T_3 concentration was significantly lower in the thermo-neutral treated group (0.95 ± 0.11 ng/ml), while it was not significantly lower in the propolis (1.260 ± 0.19 ng/ml), the synthetic vitamin C (1.14 ± 0.17 ng/ml) and vitamin E (1.64 ± 0.45 ng/ml) groups as compared to the heat stress untreated group (1.98 ± 0.31 ng/ml).

The results of the serum thyroxine (μ g/dl) level were shown in Fig. 3., which showed non signifi-

cant differences in all treated groups compared to heat stressed group. Whereas, there were significant ($p < 0.05$) differences between the heat stressed and the thermo-neutral groups.

Serum T_4 concentration in the heat stress untreated group (0.73 ± 0.13 μ g/dl) was significantly ($P \leq 0.05$) lower than thermo-neutral group (1.10 ± 0.09 μ g/dl), and was non significantly lower than vitamin E supplemented group (0.74 ± 0.09 μ g/dl), followed by (0.84 ± 0.14 μ g/dl) propolis and synthetic vitamin C (1.1 ± 0.09 μ g/dl) groups.

Discussion

The results showed that there was an improvement in hormonal indicators of heat stress in Ross broilers when propolis, asorbic acid or vitamin E was included in the diets.

Hypothalamic-pituitary-adrenal axis has an important role in behavioral and immunological responses during stress. High ambient temperature induced production and release of corticosteroids (Siegel, 1995), exerted catabolic effects (mobilization of proteins and lipids) through muscle wasting and reduces growth rate (Odedra *et al.*, 1983). In the present investigation, no differences were seen in serum corticosterone level, although it was significantly ($P < 0.05$) higher in heat stressed broilers in comparison to birds reared under thermo-neutral condition. According to Post (2003), the level of

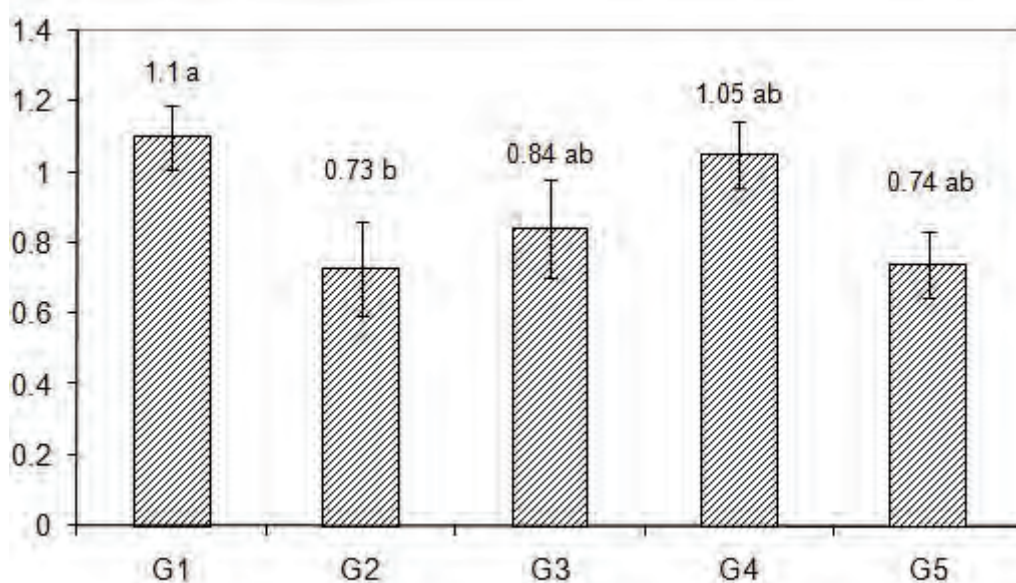


Fig. 3. Effect of treatment with Propolis (G3), ascorbic acid (G4), vitamin E (G5) on serum Thyroxin ($\mu\text{g}/\text{dl}$) level in Ross broilers reared under heat stress. Values are each mean \pm SE, ANOVA followed Duncan's multiple range test ($P < 0.05$); means having same superscripts does not differ significantly at 5% level.

body corticosterone increases as a result of stress. The increased level of glucocorticoids under stress was confirmed by many authors (Sapolsky *et al.*, 2000; Korte, 2001). Thermal stress applied in the present study increased the levels of corticosterone by an average of 67.57%. However, different results were obtained by Lin *et al.* (2004), who did not find any effect of heat stress on the levels of corticosterone. The addition of propolis at 250mg / kg diet was followed by a non significant decrease in serum corticosterone level. Our results agreed with the findings of Missima and Sforcin (2008) and Pagliarone *et al.* (2009a and 2009b) who reported that propolis treatment non significantly reduced the serum corticosterone levels in stressed animals. It could be inferred that propolis possesses biological activities such as that of antioxidants (Orhan *et al.*, 1999). The addition of ascorbic acid at 250 mg/kg diet significantly reduced the serum corticosterone level in heat stressed broilers. Our results agreed with the findings of Nockels *et al.* (1973) and Sheila and Cheryl (1978) who mentioned that, ascorbic acid has been widely used to reduce the stress in chickens, because this vitamin could decrease corticosterone level in the blood circulation. Serum concentration of ACTH was found lower with dietary vitamin C and vitamin E probably indicating a lower response to heat stress in the presence of these two vitamins in diet (Sahin *et al.*, 2002). Similarly, Kutlu and Forbes (1993) reported that heat stress tended to elevate plasma corticos-

terone concentrations, which were significantly reduced with vitamin C supplementation in broilers. Pardue *et al.* (1985a and 1985b) and McKee *et al.* (1997) also reported that supplemental vitamin C enhanced weight gain and reduced plasma corticosterone in heat-stressed chickens.

On contrary to this study findings, Sahin *et al.* (2001a and 2001b) found that heat stress (34 °C) elevated plasma corticosterone concentration, which was significantly reduced with vitamin E supplementation in the diet of Japanese quails. Moreover, Sahin *et al.* (2002) reported that increasing dietary vitamin E supplementation resulted in linear decrease in ACTH concentration.

The major hormone product of thyroid gland, T₄ is considered to be a prohormone of the more biologically active 3, 5, 3' triiodothyronine (T₃) (He *et al.*, 2000). Both T₃ and T₄ play important roles in regulating metabolism and thermogenesis in chickens (Tao *et al.*, 2006). The selective peripheral conversion of T₄ to T₃ or reverse T₃ (rT₃) is believed to play an important role in thermoregulation in domestic fowl (Rudas and Pethes, 1984). When chickens are exposed to warm temperatures, T₄ is inactivated by conversion into rT₃, whereas during cold exposure T₄ is converted into T₃, which stimulates metabolic activity. While it is generally accepted that T₃ stimulates metabolic rate and that both T₃ and T₄ concentration depressed following heat stress, this pattern is not universally observed (Scheele *et al.*, 1991; Etches *et*

al., 1995). However, the differences in the blood concentrations were more expressive for T₃ than for T₄, emphasizing that T₃ variation is more accurate in studies of thermal stress (Lawrence and Fowler, 1997). It has been suggested that thyroid activity is affected by environmental temperature (McNabb and King, 1993; Yahav *et al.*, 1997). Huston and Carmon (1962) reported the thyroid size and thyroid secretion rate decreased at high temperatures and increased at low temperatures. Jonier and Huston (1957) also reported smaller thyroid sizes at high environmental temperatures and suggested that at high temperatures the thyroid activity and subsequently metabolic rate might be reduced.

The normal value of T₃ was reported to be 0.5-4.0 ng/ml in poultry (Sturkie, 2000). Several researchers reported reduced concentrations of T₃ and T₄ in heat-stressed chickens (Heninger *et al.*, 1960; Johnson, 1981; Bowen *et al.*, 1984).

The heat stress applied in our study increased significantly ($p < 0.05$) the serum level of T₃ in heat stress birds receiving standard diets as it compared to those reared under thermo-neutral environment. Thermal stress applied in the present study increased the level of T₃ by an average of 108.4 %. Our results agree with Bobek *et al.* (1980) and Nadia (2003) who reported a significant increase in T₃ in Japanese quails exposed to 35 °C, in comparison with that exposed to room temperature at all ages of egg production. Moreover, in Japanese quails and pigeons, plasma T₃ and T₄ concentrations have been reported to increase, decrease or remain unchanged following heat stress (Bowen and Washburn, 1985). On contrary, many authors found that, under elevated temperature, the level of thyroid hormones were decreased (Williams and Njoya, 1998; Sokolowicz and Herbut, 1999). Lin *et al.* (2004) reported that heat stress to 28 days of age was associated with a decrease in T₃ level and a concurrent increase in T₄ level. However, at 38 days of rearing they did not find changes in the level of T₃ under heat stress, while T₄ concentration decreased. Also, these results disagree with that of Garriga *et al.* (2006) who found that acclimation increases the adaptive ability of birds to subsequent thermal stress by reducing the level of triiodothyronine. Williamson *et al.* (1985) suggested that, the reduction in T₃ is a consequence of the reduction in food intake as well as the increased adrenocortical activity. Also, Song *et al.* (2012)

recorded that, exposing laying hens to high ambient temperatures (32 °C) did not change the concentration of plasma T₃ and suggested that besides the reason of temperature accumulation, another possibility was that heat-exposed hens could control their body temperature by reducing feed intake, thereby eliminating the need to change metabolic rates and plasma T₃ level.

It has been reported that thyroid hormone administration stimulates heat production with increased metabolic rate resulting in reduced thermo-tolerance (Bowen and Washburn, 1985) and in increased mortality. In the present study, the addition of 250 mg/ kg diet of propolis, ascorbic acid or vitamin E non-significantly reduced the serum T₃ levels. The T₃ values were 1.98, 0.95, 1.26, 1.14 and 1.64 (ng/ml) in heat stressed group, thermo neutral, 250 mg / kg diet propolis, ascorbic acid or vitamin E, respectively, resulting in functional hypothyroidism (Mitchell and Carlisle, 1992). As a result, heat tolerance improves as thyroid function is reduced (Bowen and Washburn, 1985).

The addition of 250 mg/ kg diet of propolis non-significantly reduced the T₃ level, while, T₄ was non-significantly increased. This finding could be attributed to flavonoids (one of the main component of propolis). Flavonoids are widely distributed in plant-derived foods and possess a variety of biological activities including antithyroid effects in experimental animals and humans. The total serum T₃ concentration was decreased and the percent free T₃ increased, these changes were modest, and the serum free T₃ concentrations remained normal after administration of flavonoids (Lueprasitsakul *et al.*, 1990). Flavonoids inhibit thyroid peroxidase (TPO), the enzyme that catalyzes thyroid hormone biosynthesis (Divi and Doerge, 1996). Flavonoids inhibit thyroperoxidase activity, decreasing thyroid hormones levels thus increasing TSH. Flavonoids could also affect the availability of thyroid hormones to target tissues, by inhibiting deiodinase activity or displacing T₄ from transthyretin (TTR) leading to disturbances in thyroid hormone availability in tissues. Thus, flavonoids have been shown to interfere with many aspects of the thyroid hormones synthesis (Van der Heide *et al.*, 2003; de Souza Dos Santos *et al.*, 2011). The addition of 250 mg of propolis, had a non-significant positive effect on serum T₄ level in respect to heat stress group. This finding could be

related to explanation of Lueprasitsakul *et al.* (1990), who mentioned that the naturally occurring and synthetic plant flavonoids inhibits the binding of T₄ to human, dog and rat serum, inhibited the binding of T₄ to TTR, resulting in a decrease in the serum T₄ concentration, an increase in the percentage of serum free T₄, and an increase in the serum total free T₄ concentration. The transient elevations of serum free T₄ concentrations resulted in a significant decrease in the serum TSH concentration. These observations strongly suggest that the serum free T₄ concentration and not T₄ bound to serum TTR is biologically available to the pituitary to regulate TSH secretion and/or synthesis.

With respect to the thyroid, flavonoids have been reported to inhibit iodide uptake, exert a thiourea-like antithyroid action inhibiting TPO enzyme activity, inhibit type I and type II 5'-deiodinase as well as 5-deiodinase activity (Divi and Doerge, 1996; Ferreira *et al.*, 2002; Van der Heide *et al.*, 2003; Hamann *et al.*, 2006) and displace T₄ from serum TTR in rats (Schröder-van der Elst, 1997; Van der Heide *et al.*, 2003; Hamann *et al.*, 2006).

Both vitamin C and vitamin E have antioxidant properties, antioxidant vitamins had been proved to protect the biological membranes against the damage of reactive oxygen species (ROS) and the role of vitamin E as an inhibitor "chain blocker" of lipid peroxidation has been well established (Seyrek *et al.*, 2004). Vitamin E and ascorbate are chain breaking antioxidants, prevents lipid peroxidation due to peroxy radicals. They protect against DNA damage induced by H₂O₂ radical. Vitamin C has a paradoxical effect as it can also produce ROS by its action on transition metal ions (Lutsenko *et al.*, 2002). Frey (1991) reported that vitamin C has an ability to spare other antioxidants in relieving oxidative stress in human subjects. Vitamin C was found to assist in absorption of folic acid by reducing it to tetrahydrofolate, the latter again acts as an antioxidant. Use of folic acid is impaired when vitamin C is deficient. In the present study, the addition of 250 mg/kg diet of ascorbic acid or vitamin E had the same effect like propolis, their supplementation non-significantly reduced T₃ level and non-significantly altered T₄ level. These findings were supported by Chang *et al.* (2005), who reported that the addition of vitamin E (100 IU/kg) for the ration fed to male Cornell K strain single comb white leghorn chickens, resulted in an in-

crease in plasma T₄ concentrations, while plasma T₃ concentrations were decreased. However, Sahin *et al.* (2002) conclude that the serum concentrations of T₃ and T₄ increased as dietary vitamin E increased up to 250 mg/kg of diet, but further increases in dietary vitamin E supplementation up to 500 mg/kg of diet did not change the concentrations of T₃ and T₄. Also, supplementation of ascorbic acid in broilers has been shown to increase thyroid hormone concentration in serum (WeiLong *et al.*, 2000). These results could be due to the positive effects of ascorbic acid and vitamin E in alleviating the negative effects of heat stress.

The differences between the finding in this study and the previous work may be attributed to differences in the level of heat stress used. As in this study the birds were exposed to higher environmental temperature (38±1.4 °C).

Conclusion

Dietary supplementation of broilers with 250 mg/kg diet propolis, vitamin E or vitamin C can attenuate heat stress induced oxidative damage. These positive effects were evidenced by reduced serum corticosterone levels and improvement in thyroid hormonal levels in comparison to non-supplemented birds reared under heat stress. Moreover, supplementation with vitamin C is the most efficient antioxidant compounds. Nevertheless, further investigations will be needed for evaluating the potential beneficial effects of antioxidant activities of propolis during long-term exposure to heat stress and for characterizing more precisely the best dose for propolis to be used in broilers diet. Further studies are necessary to test this hypothesis and to elucidate the underlying mechanisms of the action.

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