Asian-Aust. J. Anim. Sci.
Vol. 21, No. 8 : 1164 - 1170
August 2008

www.ajas.info

The Effects of Dietary Turkish Propolis and Vitamin C on Performance, Digestibility, Egg Production and Egg Quality in Laying Hens under Different Environmental Temperatures

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ABSTRACT : In this study, the effects of propolis and vitamin C (L-ascorbic acid) supplementation in diets were investigated on feed intake (FI), body weight (BW), body weight gain (BWG), feed conversion rate (FCR) and digestibility and on egg production and qualities (weight, mortality, shell thickness) in laying hens exposed to heat stress. A total of 150 Hyline White Leghorn, aged 42 weeks, hens was divided into five groups of 30 hens. Chicks were randomly divided into 1 positive control, 1 control and 3 treatment groups. The chicks were kept in cages in temperature-controlled rooms at 22°C for 24 h/d (positive control, Thermoneutral, TN group) or 34°C for 9 h/d from 08.00-17.00 h followed by 22°C for 15 h (control, heat stress, HS group) and fed a basal diet or basal diet supplemented with vitamin C (250 mg/kg of L- ascorbic acid/kg of diet) or two levels of propolis (2 and 5 g of ethanol extracted propolis/kg of diet). Increased FI ($p<0.05$) and improvement in FCR ($p<0.05$), hen day egg ($p<0.05$) and egg weight ($p<0.05$) were found in Vitamin C and propolis-supplemented laying hens reared under heat stress conditions. Mortality rate was higher in the control group than TN, vitamin C and propolis groups ($p<0.05$). Digestibility of dry matter, organic matter, crude protein and ether extract improved with increasing of both dietary vitamin C and propolis ($p<0.05$). Vitamin C or propolis supplementation did not affect either the percentage shape index, yolk index or haugh unit and albumen index ($p>0.05$). However, the egg shell thickness and egg shell weight appeared to be increased in Vitamin C and propolis groups in comparison to HS group birds ($p<0.05$). In conclusion, dietary supplementation of laying hens with anti-oxidants (vitamin C and propolis) can attenuate heat stress-induced oxidative damage. These positive effects were evidenced by increased growth performance and digestibility, improvement of egg shell thickness and egg weight in comparison to non-supplemented birds. Moreover, supplementation with propolis (5 g/kg diet) was the most efficient treatment. (**Key Words :** Vitamin C, Propolis, Heat Stres, Laying Hen)

INTRODUCTION

Stress is defined as the interaction between stress factors and protective reactions. Factors causing stress include physiological factors, such as climate, environment, nutrition, and diseases, and physical conditions, such as cage density and transport (Freeman, 1987). Under stress, rapid and temporary changes occur in the body initially; with continuous stress, these are followed by permanent and irreversible changes. Finally, a decline in yield and resistance to diseases may occur. Animals under stress become ill more easily, and excess medicine may be necessary to maintain health. As a result, drug residues increase in animal products and threaten public health directly. Stock health and welfare management are key factors in animal health and food safety. For this reason, stress conditions in animals need to be examined carefully (Onbaşılar and Aksoy, 2005).

The suitable temperature for poultry is between 16-25°C (Filizciler et al., 2002; Cerci et al., 2003). Heat stress begins when the ambient temperature climbs above 25°C and is readily apparent above 30°C. Heat stress in laying hens is prompted by combinations of environmental temperature and humidity that prevent the bird's thermoregulatory process from effectively dissipating the heat produced during metabolism (Webster, 1983). High environmental temperature is the major problem faced by laying hens as well as by poultry farmers, usually in summer months. Heat stres in laying hens reduces live weight gain, feed intake, feed efficiency, production and quality of eggs and

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Received October 18, 2007; Accepted March 17, 2008

Table 1. Ingredients and chemical analyses of the basal diet of laying hen

Ingredients	Diet
Maize	65.05
Soybean meal	17.20
Sun flower meal	3.80
Fish meal	3.0
Limestone	9.0
Dicalcium phosphate	1.2
Vitamin-mineral premix[1]	0.3
Salt	0.31
Dl-methionine	0.14
Chemical analyses, dry matter (DM) basis	
Dry matter (%)	90.3
Metabolizable energy[2] (kcal/kg)	2,700
Crude protein (%)	16.0
Calcium (%)	3.90
Utilizable phosphorus (%)	0.36
Lysine (%)	0.70

[1] Vitamin and mineral premix provided per kilogram of diet: Vitamin A, 12,000 IU; kolekalsiferol 1,500 IU; vitamin E, 30 mg; vitamin K_3, 5 mg; vitamin B_1, 3 mg; vitamin B_2, 6 mg; vitamin B_6, 5 mg; vitamin B_{12}, 30 μg; Ca-D-pantotenat, 10 mg; Folic asit, 0.75 mg; D-biotin, 0.08 mg; Mn, 80 mg; Zn, 60 mg; Fe, 40 mg; Cu, 5 mg; Se, 0.15 mg; Co, 0.1 mg; I, 0.4 mg.
[b] Calculated.

increases mortality (Ciftci et al., 2005). Researchers have tried to minimize the effect of heat stress by changing the environment and diets of laying hens. Environmental approaches include increasing the airflow over birds to increase heat loss, by increasing ventilation rates or by using evaporative cooling systems in enclosed houses and lowering stocking densities. Nutritional modifications usually made are the optimization of diets to meet the altered needs of stressed birds for protein and energy and for providing some additional nutrients. Because it is expensive to cool poultry houses, methods are focused mainly on nutritional modifications. For this aim, antioxidants such as vitamin C, vitamin E and propolis are used in the poultry diet because of their anti-stress effects and also because their synthesis is reduced during heat (Tatli Seven et al., 2006; Ipek et al., 2007; Tatli Seven et al., 2008).

Vitamin C has been supplemented to diets of poultry reared under stress. In addition, several studies revealed a beneficial effect of Vitamin C supplementation on growth rate, egg production, egg shell strength and thickness in stressed laying hens and broilers (Bains, 1996; Tatli Seven, 2006).

Propolis is an adhesive, dark yellow to brown colored balsam that smells like resin. It is collected from buds, leaves and similar parts of trees and plants like pine, oak, eucalyptus, poplar, chestnut, etc. by bees and mixed with wax (Valle, 2000). Propolis supplementation is used in the poultry diet (Shalmany and Shivazad, 2006; Tatli Seven et al., 2008) In many studies conducted with propolis, positive effects like increase in feed intake (FI), body weight (BW) increase and flavonoid content, in terms of its structure, taste improvement, antioxidant and antimicrobial properties, have been reported. Anti-oxidative, 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., 2003).

Although it is known that propolis is effective in cell membrane similarly to vitamin C in oxidative stress conditions, this study aimed to determine whether propolis prevents the negative effects caused by heat stress on performance, digestibility and egg qualities similar to vitamin C. The objective of this study was to determine the possible beneficial effects of dietary propolis and vitamin C supplementation on FI, BW, body weight gain (BWG), feed conversion rate (FCR), digestibility and, particularly, on egg production and qualities (weight, mortality, shell thickness) in laying hens exposed to a chronic heat stress.

MATERIALS AND METHODS

Animals and diets

The experiment was conducted in accordance with animal welfare protocols of the Veterinary Faculty in Elazig- Turkey. A total of 150 Hyline White Leghorn, aged 42 weeks, hens was divided into five groups of 30 hens. Chicks were randomly divided into 1 thermoneutral (positive control), 1 heat stress (control) and heat stress+3 treatment groups. The chicks were kept in cages in temperature-controlled rooms at 22°C 24 h/d (Thermoneutral, TN group) or 34°C for 9 h/d from 08.00-17.00 h followed by 22°C for 15 h (heat stress, HS group) and fed a basal diet or basal diet supplemented with vitamin C (250 mg/kg of L-ascorbic acid/kg of diet, vitamin C groups) or two levels of propolis (2 and 5 g of ethanol extracted propolis/kg of diet; P2 and P5 groups, respectively). Ingredients and chemical composition of the basal diet are shown in Table 1. The basal diet was a typical layer diet containing 2,700 kcal/kg metabolisable energy (ME) and 16.20% crude protein (CP), and was calculated to meet or slightly exceed the nutrient requirements recommended by the National Research Council (NRC, 1994). Each group contained 5 hens in 6 cages. The hen house was provided with 17 h light per day. The hens were randomly assigned according to initial body weights. Feed and water were given *ad libitum*. Similar management conditions were maintained for all groups. Temperatures and humidity were recorded at a particular time daily (06.00, 12.00, 18.00 and 24.00 h within the experimental house). Average ambient relative humidity inside the hen house was 61.5±2.8%. The mean value of daily temperature in the house was 34.50±3.5°C. The

Table 2. Chemical composition assessed by GC-MS of propolis[1]

RT	Contents	% TIC
	Flavonoids	
52.49	Chrysin	5.33
53.67	Acacetin	3.02
51.66	Naringenin	2.67
	Aliphatic acids	
55.49	Decanoic acid	0.28
46.93	Octadecanoic acid	0.39
21.00	Tetradecanoic acid	0.40
51.22	Undecanoic acid	0.79
7.18	Butanedioic acid	0.77
	Aromatic acids	
26.93	Ferulic acid	0.43
24.80	Cinnamic acid	0.41
31.20	Palmitoleic acid	0.51
	Esters	
34.92	4,3 acetyloxycaffeate	0.52
36.33	Caffeic acid TMS ester	0.39
	Alcohol, terpen ve quinonee	
11.43	1-propen-1thiol	4.51
7.18	1-siklohekzen-1-methanol	4.64
28.93	Farnesol	20.64
14.97	Limonen dioxide	0.78
6.87	Glycerole	1.04
	Others	
11.43	1H-sikolpentafuran	3.17
53.43	3- hexane	1.61
56.46	Heptane	0.02
42.17	1,3 bis 5 propil benzene	0.55

RT: Retention time, minute.
[1] The ion current generated depends on the characteristics of the compound concerned and it is not a true quantitation.

experiment was carried out from June 15th to August 15th.

Performance, digestibility and egg quality

BW was recorded at the begining and end of the study to determine BW changes. FI was measured weekly. During the treatment, mortality in each group was recorded. The number of eggs and egg weight were recorded daily throughout the experiment. Random samples of 10 eggs from each treatment were collected biweekly to measure egg quality. Parameters measured for egg quality were shape index, yolk index, albumen index, Haugh unit, egg shell thickness and egg shell weight. Haugh units were calculated from the HU formula (Eisen et al., 1962) based on the height of egg-white determined with a micrometer and egg weight (Saginomiya, TLM-N1010, Japan). Shell thickness was determined by measuring the thickness mean values taken at three spots on the egg (air cell, equator, and sharp end) using a dial pipe gauge (Mitutoyo, 0.01-20 mm, Japan). From day 51 to 57, sixty birds were placed into individual battery cages and distributed into six treatments, 10 birds each, for determination of nutrient digestibility. Digestibility of nutrients was measured by collecting excrement samples twice a day. The excrement samples were oven- dried at 60°C for 48 h and then were ground for chemical analyses. Digestibility of nutrients was measured using Cr_2O_3 (0.25% of the grower diet) as indicator as described by Petry and Rapp (1971).

Laboratory analyses

Chemical analyses of the diets and excrement samples were run using international procedures of AOAC (1995). Propolis samples were collected from Elazig province (Eastern Anatolia). Hand collected propolis samples were kept desiccated in the dark until processing. Propolis samples were extracted for a week with 100 ml of 70% ethanol at room temperature to obtain the extract (Blonska et al., 2004). After filtration, the extract was evaporated by using a vacuum evaporator at 50°C. Afterwards, the extract was used in the experiment. Gas chromatography-mass spectrometry analysis were carried out to detect main components of propolis by an Agilent GC 6890 gas chromatograph, coupled to an Agilent MSD 5973 mass detector under electron impact ionization mode. The gas chromatography column used for the analysis was Zebron (ZB-1) methyl polysiloxane (30m L×0.25 mm10×0.25 μmdf). Helium was used as carrier gas at a flow rate of 10 ml/min. Propolis samples were analyzed with the column held initially at 100°C for 5 min and then increased to 150°C and kept at 150°C for 2 min. Finally, the temperature of the sample was raised to 280°C with a gradient of 2°C/min., and was kept constant at 280°C for 60 min. The injection was performed in split mode at 250°C and the peaks were identified by computer searches in commercial reference libraries. The main components of propolis samples were determined by considering their areas as percentage of the total ion current. The main compounds of propolis samples were identified and listed in Table 2. In order to estimate protein digestibility, excrement N was chemically analyzed according to the method of Terpstra and De Hart (1974).

Statistical analyses

All data were subjected to analysis of variance procedures (ANOVA) and Duncan multiple-range test (SPSS, 1999). Results were considered as significant when p values were less than 0.05.

RESULTS

The effects of supplemental vitamin C and propolis on performance, digestibility and egg qualities are shown in Table 3, 4 and 5. Birds kept at high ambient temperature (34°C) consumed less feed and gained less weight ($p<0.05$) compared with the TN group. An increase in FI ($p<0.05$), improvement in FCR ($p<0.05$), hen day egg ($p<0.05$) and egg weight ($p<0.05$) were found in Vitamin C and propolis-

Table 3. Effects of dietary supplementation with propolis and vitamin C on growth performance and egg production in laying hens reared during heat stress

	TN	HS	Vitamin-C	P-2	P-5	p
Feed intake (g/hen/d)	121.32±3.45^{a}	105.92±4.10^{d}	114.45±3.87^{bc}	110.02±5.39^{cd}	116.47±4.93^{b}	p<0.05
Body weight initial (g)	1,632.4±14.50	1,651.19±10.89	1,650.66±13.15	1,620.71±11.80	1,654.28±19.10	NS
Body weight final (g)	1,912.4±28.55^{a}	1,784.6±16.42^{c}	1,845.80±10.09^{bc}	1,804.40±25.36^{bc}	1,859.92±17.31^{ab}	p<0.05
Hen-day egg production (%)	83.25±1.50^{a}	69±3.36^{c}	78±6.05^{ab}	76±1.4^{b}	77±4.96^{b}	p<0.05
Egg weight (g/adet)	63.20±0.78^{a}	60.20±1.57^{c}	61.39±1.53^{ab}	60.45±1.76^{bc}	62.75±2.10^{ab}	p<0.05
Feed conversion rate (g feed:g egg)	1.87±0.16^{b}	2.28±0.10^{a}	2.14±0.30^{ab}	2.24±0.09^{a}	1.94±0.12^{b}	p<0.05
Mortality (%)	-	6.67±0.38^{a}	3.33±0.41^{b}	3.33±0.32^{b}	3.33±0.22^{b}	p<0.05

Results are expressed as mean±standard deviation.

$^{a-d}$ Mean values within a row with no common superscript differ significantly (p<0.05). NS: Non significant.

Table 4. Effects of dietary supplementation with propolis and vitamin C on nutrient digestibility

	TN	HS	Vitamin-C	P-2	P-5	p
Dry matter (%)	64.54±2.45^{a}	61.33±3.30^{c}	63.12±4.67^{b}	62.47±3.89^{bc}	63.85±3.52^{ab}	p<0.05
Ash (%)	32.16±2.20^{a}	28.47±2.15^{b}	29.18±1.85^{b}	29.24±2.67^{b}	29.84±1.65^{b}	p<0.05
Organic matter (%)	68.20±5.34^{a}	63.18±4.14^{c}	66.35±5.23^{a}	65.98±4.56^{ab}	66.41±4.60^{a}	p<0.05
Crude protein (%)	69.74±4.23^{a}	65.57±3.54^{c}	67.18±3.45^{b}	66.52±4.20^{bc}	67.86±2.8^{b}	p<0.05
Crude fiber (%)	1.54±0.010^{a}	1.49±0.030	1.51±0.014	1.49±0.021	1.52±0.019	NS
Ether extract (%)	71.54±3.56^{a}	67.56±5.67^{c}	69.44±4.98^{b}	69.20±4.76^{b}	69.97±5.66^{b}	p<0.05

Results are expressed as mean±standard deviation.

$^{a-c}$ Mean values within a row with no common superscript differ significantly (p<0.05). NS: Non significant.

Table 5. Effects of dietary supplementation with propolis and vitamin C on egg quality

	TN	HS	Vitamin-C	P-2	P-5	p
Shape index	68.83±3.25	67.50±2.60	67.48±2.55	67.89±2.88	67.97±1.98	NS
Yolk index	35.18±2.05	34.56±1.72	34.48±2.07	34.54±1.83	35.31±1.27	NS
Haugh unit	78.99±6.99	77.15±5.66	77.81±6.03	77.96±6.33	77.73±8.02	NS
Albumen index	6.57±0.87	6.28±0.89	6.26±0.81	6.35±0.90	6.44±0.98	NS
Egg shell thickness (mm)	0.365±0.018^{a}	0.344±0.043^{c}	0.356±0.024^{b}	0.358±0.011^{b}	0.359±0.012^{b}	p<0.05
Eggshell weight(g)	5.77±0.18^{a}	5.67±0.06^{b}	5.72±0.10^{a}	5.73±0.08^{a}	5.74±0.07^{a}	p<0.05

Results are expressed as mean±standard deviation.

$^{a-c}$ Mean values within a row with no common superscript differ significantly (p<0.05). NS: Non significant.

supplemented laying hens reared under heat stress conditions. Mortality rate was higher in the HS group than the TN, vitamin C and propolis groups (p<0.05). Digestibility of dry matter, organic matter, crude protein and ether extract improved with increasing of both dietary vitamin C and propolis (p<0.05). Vitamin C or propolis supplementation affected neither the percentage shape index, yolk index, haugh unit or albumen index (p>0.05). However, the egg shell thickness and egg shell weight appeared to be increased in Vitamin C and propolis groups in comparison to HS group birds (p<0.05).

DISCUSSION

In our study, simultaneous dietary supplementation with vitamin C and two different doses (2 and 5 g/kg diet supplementation) propolis of laying hens exposed to heat stress significantly improved performance (increases of FCR and BWG), egg qualities (production, weight, shape index, yolk index, albumen index, haugh unit, shell thickness, egg shell weight) and nutrient digestibility. Denli et al. (2005) reported that the addition of 1 g/kg propolis to the diet of quail resulted in significantly (p<0.01) better-feed efficiency as compared to control.

Propolis has been demonstrated to be an antioxidant that scavenges the free radicals generated in an organism. Anti-oxidative, cytostatic, anti-mutagenic and immunomodulatory properties of propolis are based on its rich, mainly flavonoid, phenolic acid and terpenoid contents (Kimoto et al., 1999; Prytzyk et al., 2003; Wang et al., 2003). Flavanoids show antioxidant characteristics by chelation with trace elements or radicals (Prytzyk et al., 2003; Wang, 2003). It was reported that they protect unsaturated fatty acids against the oxidants in the cell membrane similarly to ascorbate (Havsteen, 2002). On the other hand, vitamin C itself plays important roles in cellular anti-oxidant defenses, not only by reacting with all oxygen species through formation of dehydroascorbyl, a particular inert radical, but also by transferring radical equivalents from lipid phases to the aqueous compartment. In complement, ascorbate

participates in the regeneration of reduced glutatione from the oxidized form in the cytoplasm and allows tocopherol regeneration through a non-enzymatic reaction (Ciftci et al., 2005). Heat stres leads to generation of free radicals, such as $O2^-$ and HO. These free radicals can damage cell membranes by inducing lipid peroxidation of polyunsaturated fatty acids in the cell membrane (NRC, 1994). Because radical reactions are exergonic, they contribute with failure of the thermoregulation process to the increase of body temperature observed during heat stres. As a result, dietary supplementation of these two antioxidant compounds would attenuate the deleterious heat-induced- oxidative stress.

Heat stress increases the need for antioxidants, because birds can not synthesize enough ascorbate during hot conditions (Cheng et al., 1990), and dietary supplementation with high dosages of antioxidants, such as vitamin C and E, have been conducted. In our study, we observed positive effects of supplementation with vitamin C and propolis (especially 5 g/kg diet) on bird performance (growth and egg production). The effects would be more powerful if vitamin C and propolis were supplemented at more than the dosages used in the diet. Njoku and Nwazota (1989) demonstrated that high dietary vitamin C (200, 400, 600 mg/kg) supplementation significantly increased egg production in hens exposed to heat stress. Similarly, Demir et al. (1995) reported that vitamin C supplementation in feed (200 mg/kg) during heat stress increased feed intake and eggshell thickness. On the other hand, the increase in performance with propolis supplements could be linked to the palatability of propolis diets. Propolis has palatable substances like resin, wax, honey and vanillin (Shalmany and Shivazad, 2006). The higher FCR in propolis groups was connected to the high value of FI and BW increase (Buhatel et al., 1983). Furthermore, in a previous study (Ghisalberti, 1979) it was observed that when 500 ppm propolis was added to broiler feed, the BW of the propolis group increased 20% more than the control group. This could be due to the antioxidant and palatable properties of propolis. In our study, the lowest mortality rate was in vitamin C and propolis groups (Tatli Seven et al., 2008). In a previous study, it was reported that propolis stimulated the immune system and decreased mortality rate by improving immunity (Giurgea et al., 1981). Propolis was reported to have effects on immunity by increasing macrophage activity, changing microbial populations in the stomach and intestine lumen and stimulating lymphatic tissues (Taheri et al., 2005). Furthermore, antioxidant (Nagei et al., 2003; Kumazawa et al., 2004) and anti-inflammatory (Dimov et al., 1991; Borrelli et al., 2002) qualities of propolis have a significant effect on the immune system. Also, antioxidant and anti-inflammatory agents inhibit prostaglandin synthesis as anti-immune substances, thus contributing towards a better humoral response (Toma et al., 1981; Namgoong et al., 1994). The results of our study are in accordance with the literature mentioned above.

On the other hand, bioavailability of nutrients is affected by heat stress. Several studies have demonstrated that dietary supplementation with vitamin E and /or vitamin C alleviates the negative effects of heat stress on apparent nutrient digestibility. Mc Kee and Harrison (1995) also detected an improvement in FCR of broilers as a result of vitamin C supplementation during heat stress. It is well known that vitamin C improves iron assimilation by reduction of Fe3+ to Fe2+, which is better assimilated by the intestine, and thereby vitamin C improves resistance to infections. Locally, oxidative lesions leading to conformational modifications of proteins could induce pancreatic enzyme inhibition and/or dietary protein resistance to digestion. Consequently, the presence of anti-oxidants (vitamin E/or C) could partially interfere with oxidative protein denaturation and would improve digestibility of nutrients and FCR. In the present study, propolis supplementation increased nutrient digestibility (except of ash and crude fiber) according to the HS group. This may be due to palatable (Shalmany and Shivazad, 2006), antioxidant (Tatli Seven et al., 2008) and anti-immune properties (Giurgea et al., 1981). Especially, effects on performance and digestibility of Vitamin C and propolis dietary supplementation may appear more powerful under stress, because Acikgoz et al. (2006) did not observe positively effects on FI, FCR and digestibility of propolis supplementation in broilers. However, in another study (Banomi et al., 2002) propolis supplementation caused significant effects on these parameters in ducks. These different effects of propolis may be due to the dose used (Banomi et al., 2002) or relate to the study condition (stress or no stress conditions). In this present study, two antioxidant supplements (vitamin C and propolis) significantly decreased negative effects resulting from heat stress on FI, FCR and digestibility.

It is known that egg production decreases when ambient temperature goes below or above the thermo-neutral zone. At temperatures above or below the thermo-neutral zone, corticosteroid secretion increases in response to stress (Sahin and Sahin, 2001). Similarly to the results of the present study, El-Boushy et al. (1968) reported that dietary vitamin C supplementation increased egg production and egg shell strength in stressed laying hens. It has been reported that ascorbic acid plays a role in bone maturation by improving hydroxyproline production which is required for collagen formation. Accordingly, in birds, it was postulated that ascorbic acid stimulates 1,25-dihydroxycholecalciferol and together these compounds increase calcium mobilization from bones, suggesting that vitamin C has an important role in egg shell formation

(Salhin and Salhin, 2001). In the present study, vitamin C supplementation significantly increased egg shell thickness and egg shell weight. Chee et al. (2005) reported that vitamin C (200 mg/kg) supplemented to the diet for broiler breeder hens could prevent drops in egg shell quality under highly stressful environmental temperatures. This may be due to an increased calcium mobilization from bones (Salhin and Salhin, 2001).

The increased digestibility of calcium and phosphorus when propolis is added to the diet could be due to the acid derivatives, such as benzoic, 4-hydroxy-benzoic, etc., which are found in propolis (Foucher, 1982) and that favor the solubility of calcium and phosphorus salts in the diet, thus increasing the absorption of calcium. In the present study, propolis supplementation compared to the HS group significantly increased egg shell thickness and egg shell weight. This may be due to improved calcium digestibility and absorption resulting from the acid derivatives such as benzoic, 4-hydroxy-benzoic, etc., which are found in propolis (Haro et al., 2000).

In conclusion, dietary supplementation of laying hens with anti-oxidants (vitamin C and propolis) can attenuate heat stress-induced oxidative damage. These positive effects were evidenced by increases of growth performances and digestibility together with improvement of egg shell thickness and egg weight in comparison to non-supplemented birds. Moreover, supplementation with propolis (5 g/kg diet) is the most efficient treatment.

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