Effects of Propolis on Selected Blood Indicators and Antioxidant Enzyme Activities in Broilers under Heat Stress

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Abstract

In this study, we investigated the antioxidant activity of ethanol extracts of propolis (EEP) and vitamin C on biochemical indicators and antioxidant enzyme activities of broilers exposed to heat stress (at 34 °C). The experimental groups were as follows: group I (positive control) and group II (control) were fed a basal diet, group III (vitamin C) was fed a basal diet supplemented with 250 mg vitamin C as ascorbic acid/kg, group IV (EEP-0.5) was fed a basal diet supplemented with 0.5 g EEP/kg, group V (EEP-1) was fed a basal diet supplemented with 1 g EEP/kg, group VI (EEP-3) was fed a basal diet supplemented with 3 g EEP/kg. Plasma superoxide dismutase levels of positive control, control, vitamin C, EEP-0.5, EEP-1 and EEP-3 groups were found as 0.34, 1.23, 0.50, 0.90, 0.30 and 0.41 μkat/ml, respectively ($p < 0.01$). Aspartate transaminase (except for EEP-0.5 and EEP-1 groups) and alkaline phosphatase in the control group were significantly higher than those of positive control, vitamin-C and EEP-3 groups. Malondialdehyde level in plasma, liver and muscle tissues of control group were found significantly ($p < 0.05$) higher than those of positive control and EEP-3 groups. Catalase activities of blood, liver, kidney and heart were the highest in the control group. Reduced glutathione activities of plasma and liver of all groups were not significantly different from each other, whereas those of muscle, kidney and heart were significantly higher in the control group. Significantly lower levels of glutathione peroxidase were found in blood, liver and kidney tissues of the control group ($p < 0.05$), whereas those of muscle and heart were similar in all groups. The results of the present study suggest that EEP and specially EEP at the supplemented dose of 3 mg/kg diet might be considered to prevent oxidative stress in the broilers exposed to heat stress.

Propolis, antioxidant enzymes, blood, heat stress, broilers

Ambient temperature is an important factor in poultry breeding. Temperature suitable for poultry ranges between 16–25 °C (Filizciler et al. 2002; Cerci et al. 2003). Heat stress reduces feed intake, body weight gain and feed conversion (Tatli Seven 2008). At the same time, heat stress increases lipid peroxidation as a consequence of increased free radical generation. It can enhance the formation of reactive oxygen species (ROS) and induce oxidative stress in cells. Oxidative damage may be minimized by antioxidant defence mechanisms that protect the cell against cellular oxidants and repair systems that prevent the accumulation of oxidatively damaged molecules. Antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) play a vital role in protecting cellular damage from harmful effects of ROS (Altan et al. 2003). The increase in lipid peroxidation decreases antioxidants such as vitamin C and vitamin E in tissues (Tatli Seven 2008).

Vitamin C has been supplemented to diets of poultry reared under stress. In addition, several works revealed a beneficial effect of ascorbic acid supplementation on the growth rate in stressed-laying hens and broilers (Bains 1996; Tatli Seven and Seven 2008). Vitamin C supplementation leads to strengthening the antioxidative defence and a consequent decreasing of oxidative stress (Tatli Seven 2008).

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Propolis is an adhesive, dark yellow to brown coloured balsam that smells like resin. It is collected from the buds, leaves and similar parts of trees and plants like pine, oak, eucalyptus, poplar, chestnut, etc. by bees and mixed with their wax. Propolis supplementation is used in poultry diets (Tatli Seven 2008; Tatli Seven and Seven 2008). The 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. 2004). It is known that flavonoids show antioxidant characteristics to the oxidants in the cell membrane like ascorbate (Havsteen 2002). Another compound in the structure of propolis, caffeic acid phenethyl ester, blocks the production of reactive oxygen types (Hosnuter et al. 2004).

Although it is known that propolis is effective similarly to vitamin C in cell membrane in oxidative stress conditions, the current study aimed to assess whether propolis prevents the negative effects caused by heat stress on biochemical indicators and antioxidant enzyme activities that are also found with vitamin C supplementation. The study was designed to determine the effects of propolis and vitamin C on biochemical indicators and antioxidant enzyme activities, and to compare the effects on these indicators of vitamin C and propolis that have antioxidant effects in broilers under heat stress.

Materials and Methods

Animals and diets

The experiment was in accordance with animal welfare, and was conducted under the protocols of the Veterinary Faculty in Elazig, Turkey. In this study, a total of 660 one-day-old broiler chicks (Ross 308) were used. The chicks were randomly divided into 1 positive control, 1 control and 4 treatment groups. For the positive control group, sixty broilers were randomly selected and separated to three replicate groups, each containing 20 animals. The temperature was maintained at 34 °C for the first 2 days, and then decreased gradually to 21 °C (thermoneutral chamber, average 24 °C). Six-hundred broilers were randomly selected for the heat stress chamber. Three replicate groups of 40 chicks were assigned to each of the control and 4 treatment groups. They were exposed to high temperature (34 °C) for 41 days. Corn and soybean meal-based feeds were formulated according to the requirements suggested by the NRC (1994). Diets were formulated as starter (until 28 d) and finisher diets (between 28 and 41 d) (Table 1). The experimental groups were as follows; group I (positive control) and group II (control) were fed a basal diet, group III (vitamin C) was fed a basal diet supplemented with 250 mg/kg vitamin C as ascorbic acid, group IV (EEP-0.5) was fed a basal diet supplemented with 0.5 g/kg EEP, group V (EEP-1) was fed a basal diet supplemented with 1 g/kg EEP, group VI (EEP-3) was fed a basal diet supplemented with 3 g/kg EEP. Small amounts of the basal diet were first mixed with the respective amounts of vitamin C and 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 a starter diet until 21 d of age, followed by a finishing diet until 41 d of age. The diets and fresh water were offered *ad libitum*. On day 41, 18 birds (9 males and 9 females from each group) were killed by cervical dislocation. Blood samples were collected from the brachial vein of 18 birds from each group on day 41. Plasma, liver, muscle, kidney and heart samples were taken immediately.

Table 1. Composition of the experimental diets, %

Ingredients	Starter	Finishing
Corn	56.00	60.81
Soybean meal	31.70	25.25
Fish meal	6.50	5.60
Soybean oil	3.50	4.80
Limestone	1.00	0.95
Dicalcium phosphate	0.20	1.60
L-Lysine hydrochloride	0.20	0.04
Vitamin-mineral premix[1]	0.35	0.50
DL- Methionin	0.30	0.20
Sodium chloride	0.25	0.25
Calculated nutrient content		
ME, MJ/kg[2]	12.95	13.33
CP, %[3]	23.20	21.70
Calcium, %[2]	1.00	1.02
Total phosphorus, %[2]	0.56	0.59

[1]Vitamin and mineral premix provided per kilogram of diet: vitamin A, 12.000 IU; cholecalciferol 1.500 IU; vitamin E, 30 mg; vitamin K3, 5 mg; vitamin B1, 3 mg; vitamin B2, 6 mg; vitamin B6, 5 mg; vitamin B12, 30 µg; Ca-D- panthotenate, 10 mg; folic acid, 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

[2] Based on NRC (1994) feed composition tables

[3] Analysed (AOAC, 1990)

Table 2. Chemical composition assessed by GC-MS of EEP[a]

RT (min)	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-cyklohexene-1-methanol	4.64
28.93	Farnesol	20.64
14.97	Limonene dioxide	0.78
6.87	Glycerole	1.04
	Others	
11.43	1H-cyclopentafuran	3.17
53.43	3- hexane	1.61
56.46	Heptane	0.02
42.17	1,3 bis 5 propylbenzene	0.55

RT: Retention time

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

Sample collection and biochemical assays

Propolis samples were collected from the Elazig Province (East Anatolia). Hand-collected propolis samples were kept desiccated in dark until the processing. Collected propolis was extracted for a week with 100 ml of 70% ethanol at room temperature to obtain the extract. After filtration, the extract was evaporated using a vacuum evaporator at 50 °C and then used in the experiment. Gas chromatography-mass spectrometry was carried out to detect main components of propolis by the Agillent GC 6890 gas chromatograph coupled to the Agillent MSD 5973 mass detector under electron impact ionization. The chromatographic column for the analysis was Zebron (ZB-1) methyl polysiloxane column (30 m L × 0.25 mm 10 × 0.25 mm df). The carrier gas used was helium at a flow rate of 10 ml/min. The 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, the temperature was increased to 280 °C with a 2 °C/min heating ramp, and the temperature was kept at 280 °C gradually for 60 min for sample. The injection was performed in a 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 the propolis sample were identified and are listed in Table 2.

The broilers in control and treatment groups were kept under the same environmental conditions. The crude protein of the diet was determined according to AOAC (1990). Blood samples were taken into tubes containing anticoagulant (2% sodium oxalate). The samples were centrifuged at 200 *g* for 5 min at +4 °C; then the plasma was removed immediately and stored at -20 °C until analyzed. Plasma biochemical indicators were measured using an auto analyzer (Olympus AU 600, Japan). Tissue specimens (liver, muscle, kidney, heart) were rinsed with saline to remove the blood. The homogenization of tissues was carried out in a Teflon-glass homogenizer with a buffer containing 1.15% KCl to obtain 1 : 10 (w/v) whole homogenate. The homogenates were centrifuged at 18.000 *g* (+4 °C) for 15 min to determine malondialdehyde (MDA), reduced glutathione (GSH) concentrations, CAT and GSH-Px activities.

The SOD activity was measured using the RANSOD kit. The role of SOD is to accelerate the dismutation of the toxic superoxide radical, produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. Plasma MDA concentration, the end product of lipid peroxidation, was measured according to the method of Satoh (1978). MDA contents of tissue homogenates were assayed spectrophotometrically according to the method of Ohkawa et al. (1979). MDA concentrations in plasma and tissue were expressed as nmol/ml and nmol/mg protein tissue, respectively. The CAT activity was estimated by measuring the breakdown of H_2O_2 at 240 nm according to the method of Aebi (1984) and expressed as k/g protein in tissues. Tissue GSH concentration was measured by an assay using the dithionitrobenzoic acid recycling method described by Ellman (1959) and was expressed as nmol/ml. Tissue protein contents were determined by the method of Lowry (1951). The GSH-Px activity was determined using the method of Beutler (1975), which records the disappearance of NADPH at 340 nm. The action of GSH-Px is to reduce H_2O_2, with coupled oxidation of NADPH. The procedure of analysis performed was based on the oxidation of GSH by GSH-Px coupled to the disappearance of NADPH by glutathione reductase measured at 37 °C and 340 nm and were expressed as U/g protein.

Table 3. The SOD activities and some biochemical indicators of the study groups (n = 18)

	Positive Control	Control	Vitamin C	EEP-0.5	EEP-1	EEP-3	P
SOD (µkat/ml)	0.34 ± 0.05^{c}	1.23 ± 0.12^{a}	0.50 ± 0.07^{c}	0.90 ± 0.06^{b}	0.30 ± 0.02^{c}	0.41 ± 0.03^{c}	**
Glucose (mmol/l)	11.12 ± 0.35	13.30 ± 0.34	12.88 ± 0.40	12.47 ± 0.30	13.82 ± 0.26	12.67 ± 0.27	NS
Albumin (g/L)	11.0 ± 0.4	12.0 ± 0.5	11.6 ± 0.3	11.0 ± 0.5	11.0 ± 0.5	11.3 ± 0.8	NS
Total Protein (g/l)	28.4 ± 1.50	29.6 ± 2.90	28.0 ± 1.00	26.6 ± 1.40	28.0 ± 1.00	29.0 ± 2.50	NS
Total Cholesterol (mmol/l)	2.38 ± 0.34	3.13 ± 0.40	3.42 ± 0.50	2.92 ± 0.40	2.62 ± 0.42	2.43 ± 0.36	NS
VLDL Cholesterol (mmol/l)	2.44 ± 0.41	3.13 ± 0.40	3.42 ± 0.50	2.92 ± 0.40	2.62 ± 0.42	2.43 ± 0.36	NS
Triglyceride (mmol/l)	0.36 ± 0.04	0.40 ± 0.08	0.38 ± 0.14	0.35 ± 0.06	0.42 ± 0.07	0.36 ± 0.05	NS
AST (µkat/l)	3.66 ± 0.32^{c}	5.90 ± 0.38^{a}	3.90 ± 0.50^{b}	4.87 ± 0.35^{ab}	4.80 ± 0.54^{ab}	4.42 ± 0.27^{b}	*
ALT (µkat/l)	0.028 ± 0.01	0.035 ± 0.005	0.027 ± 0.01	0.03 ± 0.005	0.033 ± 0.003	0.025 ± 0.006	NS
Alkaline phosphatase (µkat/l)	23.00 ± 1.64^{b}	67.47 ± 4.88^{a}	23.79 ± 1.85^{b}	28.90 ± 2.10^{b}	30.94 ± 2.94^{b}	30.56 ± 1,89^{b}	**
Potasium(mmol/l)	4.88 ± 0.34	6.66 ± 7.03	6.93 ± 0.27	6.60 ± 0.45	5.93 ± 0.38	6.26 ± .0.03	NS
Sodium (mmol/l)	146.20 ± 2.25	148.33 ± 3.48	145.33 ± 0.88	147.33 ± 1.20	154.66 ± 3.28	153.33 ± 5.45	NS
Chlorine(mmol/l)	111.56 ± 1.87	114.66 ± 3.17	111.66 ± 0.33	116.00 ± 1.52	120.66 ± 2.66	120.33 ± 5.45	NS

NS: Non-significant, *$p < 0.05$, **$p < 0.01$, a-c: Mean values with different superscripts within a row differ significantly

Statistical analysis

All values were presented as means ± SEM. Differences between group means were calculated by one-way analysis of variance (ANOVA) and post-hoc Duncan test used by SPSS/PC computer program (SPSS 1999). Results were considered significant at $p < 0.05$.

Results

Plasma SOD levels of positive control, control, vitamin C, EEP-0.5, EEP-1 and EEP-3 groups were found as 0.34, 1.23, 0.50, 0.90, 0.30 and 0.41 µkat/ml, respectively ($p < 0.01$) (Table 3). It was obvious that plasma SOD activity was affected by heat exposure. Plasma SOD activity was significantly increased in the control group compared to positive control and other supplement groups ($p < 0.01$) (Table 3). The values of plasma glucose, albumin, total protein, total cholesterol, VLDL, triglyceride, aspartate transaminase, alanine transaminase, alkaline phosphatase, potassium, sodium and chlorine of groups were presented in Table 3. The glucose, albumin, total protein, total cholesterol, VLDL cholesterol, triglyceride, alanine transaminase, potasium, sodium, chlorine in blood were not significantly influenced by heat exposure (Table 3). Aspartate transaminase (AST) (except for EEP-0.5 and EEP-1 groups) and alkaline phosphatase (ALP) in the control group were significantly higher than those of positive control, vitamin C and EEP-3 groups ($p < 0.01$).

MDA level in plasma, liver and muscle tissues of control group were found significantly higher than those of positive control and EEP-3 groups. MDA levels of kidney and heart were similar in all groups (Table 4). The MDA activity was significantly increased in plasma,

Table 4. MDA levels of plasma (nmol/ml) and some tissues (nmol/mg protein) of the study groups (n = 18)

	Positive Control	Control	Vitamin C	EEP-0.5	EEP-1	EEP-3	*P*
Plasma	11.57 ± 0.94^{b}	16.31 ± 1.08^{a}	13.40 ± 0.69^{b}	13.56 ± 0.66^{ab}	14.60 ± 0.82^{ab}	12.88 ± 1.23^{b}	*
Liver	67.43 ± 2.23^{c}	93.18 ± 2.98^{a}	83.06 ± 4.10^{ab}	93.10 ± 3.43^{a}	84.27 ± 5.77^{ab}	76.82 ± 3.86^{b}	**
Muscle	94.24 ± 2.57^{c}	177.50 ± 11.81^{a}	139.10 ± 23.61^{ab}	150.04 ± 20.81^{ab}	106.85 ± 22.80^{b}	104.20 ± 20.0^{b}	**
Kidneys	106.586 ± 9.50	117.17 ± 8.55	109.26 ± 2.85	116.37 ± 6.53	112.40 ± 6.58	115.54 ± 5.46	NS
Heart	0.40 ± 0.025	0.46 ± 0.049	0.46 ± 0.030	0.47 ± 0.043	0.40 ± 0.019	0.43 ± 0.045	NS

NS: Non-significant, $*p < 0.05$, $**p < 0.01$, $^{a\text{-}c}$: Mean values with different superscripts within a row differ significantly

Table 5. CAT activities (kat/g protein) in blood (kat/hHb) and some tissues of the study groups (n = 18)

	Positive Control	Control	Vitamin C	EEP-0.5	EEP-1	EEP-3	P
Blood	5.10 ± 0.45^{b}	8.57 ± 1.10^{a}	6.60 ± 0.71^{ab}	8.53 ± 0.67^{a}	6.51 ± 0.52^{ab}	5.20 ± 0.65^{b}	**
Liver	52.90 ± 18.76^{c}	91.01 ± 2.78^{a}	57.91 ± 10.57^{bc}	92.42 ± 7.23^{a}	83.04 ± 3.99^{ab}	62.33 ± 11.50^{b}	**
Kidneys	50.09 ± 4.85^{b}	67.18 ± 4.54^{a}	54.70 ± 5.01^{b}	59.87 ± 2.87^{ab}	56.62 ± 8.69^{ab}	51.18 ± 3.72^{b}	*
Heart	30.24 ± 9.24^{c}	68.08 ± 9.21^{a}	45.15 ± 4.52^{abc}	66.70 ± 7.63^{a}	49.31 ± 9.84^{ab}	34.45 ± 10.50^{bc}	*

$*p < 0.05$, $**p < 0.01$, a-c: Mean values with different superscripts within a row differ significantly

liver and muscle by heat exposure.

CAT activities of blood, kidney and heart were the highest in the control group. The CAT activity of EEP-3 group was found significantly lower than that of the control group (Table 5). Its CAT activities for all tissues were found nearest to the positive control group exposed to normal temperature.

GSH activities of plasma of all the groups were not significantly different from each other, whereas those of muscle, kidney and heart were significantly higher in the control group (Table 6). Liver GSH activity was obviously affected by heat exposure, but EEP and vitamin C supplementations were not sufficient to decrease its activity. Liver GSH activity of the positive control group was the least when compared to the other groups.

Significantly lower levels of GSH-Px were found in blood, liver and kidney tissues of the control group ($p < 0.05$), whereas those of muscle and heart were similar in supplement groups. GSH-Px activities of positive control and EEP-3 groups were found the highest among the groups (Table 7).

Discussion

The effects of supplementations of reference antioxidant vitamin C and EEP affected antioxidant on some blood indicators on level of MDA, and the activities of SOD, CAT, GSH, and GSH-Px in broilers exposed to heat were determined.

We found that the glucose, albumin, total protein, total cholesterol, VLDL cholesterol, triglyceride, alanine transaminase, potassium, sodium, chlorine in blood were not influenced by the vitamin C and EEP treatments. Biavatti et al. (2003) reported that propolis had no influence on biochemical indicators including glucose, creatinine, cholesterol, triglyceride, AST and ALT. However, different studies indicated that propolis aleviated too high blood lipid, high total cholesterol and arteriosclerosis (Akgul et al. 1997; Burdock 1998; Castaldo and Capasso 2002). In the present study, it was found that AST and ALP levels were significantly decreased in positive control, vitamin C and EEP

Table 6. GSH activities in blood (mmol·100 ml^{-1}) and some tissues (nmol·100 ml^{-1}) of the study groups (n = 18)

	Positive Control	Control	Vitamin C	EEP-0.5	EEP-1	EEP-3	P
Plasma	1310.01 ± 122.1	1586 ± 104.03	1451.1 ± 77.1	1542.3 ± 88.23	1509.1 ± 77.14	1342.1 ± 59.6	NS
Liver	344.08 ± 87.0^{b}	515.04 ± 93.2^{a}	461.11 ± 110.0^{a}	484.2 ± 120.3^{a}	440.0 ± 48.1^{a}	425.06 ± 97.09^{a}	NS
Muscle	326.1 ± 48.55^{b}	825.12 ± 116^{a}	363.23 ± 52.05^{b}	586.41 ± 107^{ab}	348.0 ± 43.1^{b}	410.22 ± 62.1^{b}	**
Kidney	228.24 ± 60.05^{b}	501.13 ± 108^{a}	495.15 ± 68.12^{a}	306.1 ± 46.0^{ab}	223.05 ± 41.2^{b}	404.04 ± 48.20^{ab}	*
Heart	156.1 ± 39.06^{b}	298.22 ± 23.0^{a}	262.12 ± 28.1^{a}	276.3 ± 29.2^{a}	258.2 ± 22.0^{a}	171.06 ± 26.4^{b}	*

NS: Non-significant, *$p < 0.05$, **$p < 0.01$, a.b: Mean values with different superscripts within a row differ significantly

Table 7. GSH-Px activities in blood (µkat/gHb) and some tissues (µkat/g protein) of the study groups (n = 18)

	Positive Control	Control	Vitamin- C	EEP-0.5	EEP-1	EEP-3	P
Blood	0.66 ± 0.07^{a}	0.39 ± 0.06^{b}	0.58 ± 0.07^{a}	0.46 ± 0.04^{b}	0.56 ± 0.08^{a}	0.61 ± 0.10^{a}	*
Liver	0.68 ± 0.07^{a}	0.20 ± 0.04^{c}	0.51 ± 0.06^{b}	0.51 ± 0.06^{b}	0.50 ± 0.07^{b}	0.54 ± 0.11^{b}	*
Muscle	0.58 ± 0.07^{a}	0.61 ± 0.05^{c}	0.66 ± 0.17^{b}	0.64 ± 0.10^{b}	0.56 ± 0.01^{b}	0.55 ± 0.06^{b}	NS
Kidneys	0.50 ± 0.04^{a}	0.13 ± 0.02^{c}	0.35 ± 0.03^{b}	0.27 ± 0.10^{b}	0.42 ± 0.09^{b}	0.37 ± 0.06^{b}	*
Heart	1.90 ± 0.12^{a}	1.74 ± 0.13^{c}	1.85 ± 0.20^{b}	1.80 ± 0.24^{b}	1.77 ± 0.39^{b}	1.85 ± 0.15^{b}	NS

NS: Non-significant, *$p < 0.05$, a-c: Mean values with different superscripts within a row differ significantly

groups compared to those in the control group. Zaidi et al. (2005) reported that the AST activity in blood significantly increased under stress. The AST findings may be an evidence for vitamin C and EEP decreasing the stress of broilers, in consistency with the previous study (Zaidi et al. 2005; Yousef et al. 2006). Once again, Kolankaya et al. (2002) reported that the plasma AST level was decreased by supplementary propolis. The results of the present study were in agreement with those reported by Kolankaya et al. (2002). Similarly, the ALP activity in blood was significantly increased in the control group compared to the other groups ($p < 0.01$). This can be explained by the heat stress. Likewise, it is noted that in studies related to stress, oxidative damage increased the ALP activity (Kaur et al. 2006; Manna et al. 2006; Yousef et al. 2006).

Heat stress is an important stressor resulting in the reduced welfare of birds. Heat stress increased lipid peroxidation as a consequence of increased free radical generation. The rise of lipid peroxidation increases the MDA level in blood and tissues (Okutan et al. 2005; Ates et al. 2006). In this study, it was found that plasma, liver and muscle MDA levels were significantly decreased in positive control, vitamin C and EEP-3 groups compared to those in the control. It may be considered that dietary vitamin C and a high dose of EEP (EEP-3 group) decreased lipid peroxidation. Besides, 3 g/kg dietary EEP was found more effective than vitamin C, on especially liver and muscle MDA levels. Likewise, Okonenko et al. (1988**)** reported that propolis had more pronounced antioxidant action compared to that of vitamin E that has a similar activity to vitamin C.

Living organisms are able to adapt to oxidative stress by inducing the synthesis of antioxidant enzymes and damage removal/repair enzymes (Davies 1995). Antioxidant enzyme activities such as SOD and CAT in lipid peroxidation may

sometimes decrease (Wohaieb and Godin 1987; Ozkaya et al. 2002) or increase (Huang et al. 1999; Aliciguzel et al. 2003). In the present study, the increase of antioxidant enzyme activities such as SOD, CAT and GSH may be considered as a protective mechanism against heat-induced free radical production and lipid peroxidation. Exposing birds to heat stress resulted in a significant increase in SOD and CAT (Altan et al. 2003). Moreover, significant differences between enzymes were obtained in antioxidant enzyme responses to heat treatment. A similar response has been reported in many human diseases, in which MDA concentrations increased concomitantly with an increase in antioxidant enzyme activities. McArdle and Jackson (2000) have also demonstrated a significant increase in free radical production together with an increase in the expression of antioxidant enzymes during a period of non-damaging exercise. These increases in antioxidant enzyme activities have been considered as a protective response against oxidative stress (Altan et al. 2003). In a previous study, Okutan et al. (2005) investigated the effects of caffeic acid phenethyl ester (CAPE), which is a component of propolis, on lipid peroxidation and antioxidant enzymes in a diabetic rat heart. They found that in the untreated diabetic group, the SOD activities and CAT levels were significantly decreased, while the GSH-Px activity was increased in the CAPE-treated diabetic rats compared to those observed in untreated diabetic rats ($p < 0.0001$ and $p = 0.016$, respectively). The findings reported by Okutan et al. (2005) were in agreement with our results. The GSH-Px activities of blood, liver and kidney in the control group were significantly reduced, while SOD, CAT and GSH activities were increased in blood and some tissues in the control group. This may be explained by the activity of GSH-Px in inhibition of increased free radicals in tissues (Nakazawa et al. 1996). In the present study, levels of MDA, antioxidant enzymes (SOD and GSH-Px) and GSH of blood and some organs were found similar in the groups. It can be speculated that there was no correlation of antioxidant enzyme activities between tissues (Irmak et al. 2003). Similarly, Okutan et al. (2005) reported that there is no consensus in the level of antioxidant enzymes of many organs in diabetic rats.

It can be concluded that heat stress in broilers increases oxidative stress in blood and tissues. Generally, it was found nearest to values of vitamin C and EEP-3 groups compared with those of the positive control group. EEP decreased lipid peroxidation and regulated antioxidant enzyme activities in the broilers exposed to heat stress. The protective role of EEP might be related to its antioxidant effect. The results of this study suggest that EEP and especially EEP at the supplemented dose of 3 mg/kg diet might be considered in the prevention of oxidative stress in broilers exposed to heat stress.

Vliv propolisu na vybrané biochemické ukazatele stanovované v krvi, a aktivitu antioxidačních enzymů brojlerových kuřat vystavených tepelnému stresu

V této studii byla sledován vliv antioxidační aktivity etanolových extraktů propolisu (EEP) a vitamínu C na biochemické indikátory a aktivitu antioxidačních enzymů u brojlerů vystavených tepelnému stresu (34 °C). Experimentální skupiny byly následující: skupina I (pozitivní kontrola) a skupina II (negativní kontrola) krmená základní dietou, skupina III (vitamin C) krmená základní dietou doplněnou o 250 mg vitaminu C formou kyseliny askorbové/kg, skupina IV (EEP-0.5) krmená základní dietou s přídavkem 0.5 g EEP/kg, skupina V (EEP-1) krmená základní dietou s přídavkem 1 g EEP/kg, skupina VI (EEP-3) krmená základní dietou doplněnou o 3 g EEP/kg. Aktivita superoxiddismutázy v krevní plazmě pozitivních kontrol, negativních kontrol, skupiny III, IV, V a VI byly naměřeny takto: 0,34, 1,23, 0,50, 0,90, 0,30 a 0,41 μkat/ml s průkazností ($p < 0.01$). Aktivita aspartátaminotransferázy (AST) u všech skupin kromě IV a V, a aktivita alkalické fosfatázy (ALP) v negativní kontrole byla signifikantně vyšší než u pozitivních kontrol, skupině III a VI. Obsah malondialdehydu v krevní plazmě, jaterní a svalové tkáni u kuřat kontrolní skupiny (skupina II) byla signifikantně ($p < 0.05$) zvýšená ve srovnání s pozitivní kontro-

lou a skupinou VI. Nejvyšší hodnoty enzymatické aktivity katalázy v krvi, jaterní tkáni, v ledvinách a srdci byly naměřeny v negativní kontrole. Redukovaný glutathion v krevní plazmě a v jaterní tkáni kuřat všech experimentálních skupin nebyl významně změněný, avšak signifikantně vyšší hladiny GSH byly zjištěny ve svalech, ledvinách a myokardu u negativních kontrol. Průkazně nižší aktivita glutathionperoxidázy byla zjištěna v krvi, játrech a ledvinách u brojlerů kontrolní skupiny II ($p < 0.05$), zatímco její aktivita v kosterní svalovině a myokardu byla srovnatelná ve všech skupinách. Z výsledků této studie vyplývá, že suplementace krmiva brojlerů výtažky z propolisu, konkrétně v dávce 3 mg/kg krmiva, hraje pozitivní úlohu v prevenci oxidačního stresu brojlerů vystavených účinkům tepelného stresu.

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