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The Effects of Propolis on Biochemical Parameters and Activity of Antioxidant Enzymes in Broilers Exposed to Lead-Induced Oxidative Stress

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ABSTRACT : This study aimed to determine the effects of vitamin C and propolis-supplemented feeds on some blood parameters, lipid peroxidation, and activities of some antioxidant enzymes in broilers exposed to oxidative stress. 360 three-day-old broiler chicks (Ross 308) were randomly divided into four treatment groups each containing 90 animals, including six replicate groups for each treatment. The experimental groups were designated for a 3-42 days period as follows: no supplement to basal ration (Control-Group I); supplement of 500 ppm vitamin C and 200 ppm lead (as lead acetate) to basal ration (Group II); supplement of 1 g/kg propolis and 200 ppm lead (as lead acetate) to basal ration (Group III); and supplement of 200 ppm lead (as lead acetate) to basal ration (Group IV). The highest TG level (86.83 mg/dl) was observed in the lead supplemented group; however, the lowest aspartate aminotransferase (SGOT) level (90.71 IU/L) was observed in the control group ($p<0.05$). The addition of lead increased the plasma malondialdehyde (MDA) level ($p<0.01$) compared to other treatments. However, the addition of vitamin C and propolis decreased the plasma MDA level close to control levels. The highest erythrocyte superoxide dismutase (SOD) activity was observed in the lead addition group ($p<0.01$) while no significant differences were observed for SOD activities of the control, vitamin C +lead, and propolis+lead groups. The plasma reduced glutathione (GSH) activity of the control (2.30 μmol/ml) was significantly lower than the lead administered group (6.20 μmol/ml) ($p<0.01$); while this parameter was determined to be similar to other groups. No significant differences were observed between groups for liver GSH activity, but heart GSH activity of the control was significantly higher in comparison to other treatments ($p<0.05$). To obtain similar antioxidant effects, it is recommend that using propolis (1 g/kg) and vitamin C (500 mg/kg) supplementation in broiler diets may overcome the adverse effects of oxidative stress originating from dietary lead. (**Key Words :** Broiler, Lead, Oxidative Stress, Propolis, Blood, Malondialdehyde, Antioxidant Enzymes)

INTRODUCTION

Increasing environmental pollution because of industrial development, and the effects on human and animal health of the heavy metals and metal compounds because of these major environmental pollutants, constitutes an increasingly important area of interest in recent years. Environmental exposure to lead and lead-containing compounds is detrimental to human health (especially the health of children) and is a critical ecological problem in the modern world. The human organism is primarily exposed to lead via food (about 85%). Food and raw materials for the food industry may be contaminated by lead transferred from soil, water, the atmosphere, and the fodder of agricultural animals (Lysenko, 2005).

As a major environmental pollutant, lead causes adverse effects, such as the production of reactive oxygen species (ROS), disruption of tissue oxidant/antioxidant balance, and alteration of lipid metabolism (Ahamed and Siddiqui, 2007). Reactive oxygen species (ROS) are essential for proper cell functioning and are widely produced during normal cell metabolism. Low levels of ROS are necessary for many cell-signaling procedures. Under normal physiological conditions, a balance exists between the levels of ROS produced during cellular metabolism and the levels of endogenous antioxidants, which serve to protect tissues from oxidative damage. Imbalance or loss of cellular redox

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homeostasis results in oxidative stress, causing severe damage to cellular components (Sies, 1991). An excessive level of ROS leads to a variety of pathological conditions, including lipid peroxidation, apoptosis, and tissue damage. Lipid peroxidation can compromise the integrity of cell membranes and increase cell membrane fluidity, which adversely affects immune responses**,** and lipid peroxidation deactivates the membrane-bound receptors and enzymes (Bendich, 1993). In order to protect against lipid peroxidation and oxidative damage, all living organisms have evolved an interdependent antioxidant system that includes enzymatic and non-enzymatic components in the liver (Ohtsuka et al., 1998) and erythrocytes (Orzechowski et al., 2000). The major antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) reduced glutathione (GSH), melatonine, ceruloplasmin (Cp), and albumin are non-enzymatic antioxidants (Halliwell and Gutteridge, 1986). Antioxidant enzymes play a vital role in protecting cellular damage from the harmful effects of ROS (Altan et al., 2003). In addition, the increase in lipid peroxidation decreases with the addition of some antioxidant matters.

Vitamin C has been supplemented to the 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 defenses and a consequent decrease of oxidative stress (Tatli Seven, 2008). In studies with propolis and other antioxidants, it has been reported that propolis can relieve the adverse effects of lipid peroxidation and free radical formation (Ohkawaet al., 1979; Tatli Seven et al., 2009). Flavonoids and caffeic acid phenethyl ester (CAPE), which are components of propolis, are reported to be antioxidant matters protecting the cell membranes from lipid peroxidation (Havsteen, 2002; Hosnuter et al., 2004). Propolis is an adhesive, dark yellow to brown colored 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, and so on by bees and mixed with their wax. Propolis supplementation is used in poultry diets (Tatli Seven, 2008; Tatli Seven and Seven, 2008; Tatli Seven et al., 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).

This study was therefore designed to determine the effects of propolis on biochemical parameters, MDA levels and some antioxidant enzyme activities in broilers under oxidative stress originating from lead.

MATERIALS AND METHODS

Animals, diet, and experimental design

The experiment was conducted in accordance with animal welfare and under protocols by the Veterinary Faculty in Hatay-Turkey. A total of 360 three-day-old broiler chickens (Ross 308) were used in the feeding trail that lasted until the birds reached 42 days old. The experimental animals were divided into four groups comprising one control group, and three experimental groups each consisting of 90 chickens. All groups were also divided six replicate groups containing 15 broiler chicks each. The birds were given *ad libitum* access to feed and water. A lighting schedule of 23 L:1 D was imposed throughout the experimental period. Ambient temperature was gradually decreased from 32°C on day 1 to 22°C at the end of the experiment. Newcastle disease vaccination was performed on day 10, whereas the Gumboro vaccination took place on day 18. The basal diet (Table 1) was formulated according to NRC (1994) and analyzed by the AOAC (1995). Two phases were applied during the experiment: a starter (0-21 d) and finisher (21-42 d).

The experimental groups were designed as (1-) no supplementation to basal ration (Control-Group I); (2-) supplementation of 500 ppm vitamin C and 200 ppm lead (as lead acetate) to basal ration (Group II); (3-) supplementation of 1 g/kg propolis and 200 ppm lead (as lead acetate) to basal ration (Group III); and (4-) supplementing of 200 ppm lead (as lead acetate) to basal ration (Group IV) for a period of 3-42 days.

On day 42, total 24 birds (one birds from in each subgroup) were killed by cervical dislocation. Blood samples were collected from brachial vein before cervical dislocation. Plasma, liver and heart samples were taken immediately.

Sample collection and biochemical assays

Propolis samples were collected from Elazig province (Eastern Anatolia). Hand-collected propolis samples were kept dried 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 was carried out to detect the 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

Table 1. Composition of the basal diet (%)

Feed components			Start (3-21 days)	Finish (22-42 days)
Maize			55.71	61.21
Soybean meal (44%)			29.00	27.00
Fish meal (64%)			7.60	4.00
Vegetable oil			4.50	4.50
Limestone			1.20	1.30
Dicalcium phosphate			1.20	1.20
Vitamin-mineral premix[1]			0.35	0.35
DL-methionine			0.19	0.19
Salt			0.25	0.25
Nutrients content	Analysis results (%)	Dry matter	90.48	90.73
		Crude protein	22.6	19.8
		Crude ash	6.1	5.7
		Ether extract	7.53	7.40
		Calcium	1.00	0.98
		Total phosphorus	0.68	0.64
	Calculation results (%) [2]	Metabolizable energy (kcal/kg)	3,138	3,177
		Crude fiber	3.03	3.00
		Met.+Cysteine	0.94	0.85
		Lysine	1.42	1.18

[1] Vitamin and mineral premix provided per kilogram of diet: Vitamin A, 12,000 IU; cholecalciferol 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 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] Calculated by NRC (1994).

chromatography column was Zebron (ZB-1) methyl polysiloxane (30 ml×0.25 mm 10×0.25 m df). Helium was used as a carrier gas at a flow rate of 10 ml/minute. Propolis samples were analyzed with the column held initially at 100°C for 5 minutes and then increased to 150°C and kept at 150°C for 2 minutes. Finally, the temperature of the sample was raised to 280°C with a ramp rate of 2°C/minute, and it was kept constant at 280°C for 60 minutes. 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 a percentage of the total ion current. The main compounds of propolis samples were identified and listed in Table 2.

Blood and tissue analysis

A total of 24 chickens, six from each group, were selected. Blood samples were taken into tubes containing anticoagulant (2% sodium oxalate). The samples were centrifuged at 200 g for 5 minutes at +4°C; then the plasma was removed immediately and stored at -20°C until analyzed. Biochemical indicators of blood, such as glucose, albumin, total cholesterol, triglyceride (TG), aspartate aminotransferase (SGOT), alanine amino transferase (SGPT), high-density-lipoprotein (HDL), and low-density-lipoprotein (LDL) were measured using an auto analyzer (Olympus AU 600, Japan).

Tissue specimens (liver and heart) were rinsed with

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

RT		Contents	% TIC
52.49	Flavonoids	Chrysin	5.33
53.67		Acacetin	3.02
51.66		Naringenin	2.67
55.49	Aliphatic acids	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
26.93	Aromatic acids	Ferulic acid	0.43
24.80		Cinnamic acid	0.41
31.20		Palmitoleic acid	0.51
34.92	Esters	4,3 acetyloxycaffeate	0.52
36.33		Caffeic acid TMS ester	0.39
11.43	Alcohol, terpene and quinonee	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
11.43	Others	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.
TIC = The ion current generated depends on the characteristics of the compound concerned and it is not a true quantitation.

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 minutes to determine malondialdehyde (MDA), reduced glutathione (GSH) concentrations and CAT activities.

SOD activity was measured by using 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 and tissue homogenates were assayed spectrophotometrically according to the method of Placer et al. (1966). MDA concentrations in plasma and tissue were expressed as nmol/ml and nmol/mg protein tissue, respectively. CAT activity was estimated by measuring the breakdown of H_2O_2 at 240 nm according to the method of Aebi (1984). CAT activity was 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 et al. (1951).

Statistical analysis

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

RESULTS

Biochemical parameters of the study groups are given in Table 3. The biochemical parameters examined, except TG and SGOT, were not affected by administration. The highest TG level (86.83 mg/dl) was observed in the lead supplemented group; however, the lowest SGOT level (90.71 IU/L) was observed in the control group ($p<0.05$). When the MDA levels of plasma, liver, and heart were examined (Table 4), it was determined that the lead addition increased the plasma MDA level ($p<0.01$) compared to others. But the addition of vitamin C and propolis decreased the plasma MDA level close to control levels. The lowest MDA level in liver (0.88 nmol/mg) was observed in the control group ($p<0.05$).

The highest erythrocyte SOD activity was observed in the lead addition group ($p<0.01$) while no significantly differences were observed for SOD activities of control, vitamin C+lead, and propolis+lead groups. On the other hand, the CAT activities of erythrocyte and heart were significantly lower ($p<0.01$) in the control group while the CAT activities of heart, liver, and erythrocyte were similar in the vitamin C and propolis added groups (Table 3).

When the GSH activity of plasma, liver, and heart were examined (Table 6), it was determined that the plasma GSH activity of the control group (2.30 μmol/ml) was significantly lower than the lead administrated group (6.20 μmol/ml) ($p<0.01$); while this parameter was determined to be similar to other groups. No significant differences were observed between groups for the liver GSH activity but the heart GSH activity of the control group was determined to

Table 3. Biochemical parameter values of research groups (n = 6)

	Control	Vitamin C+Pb	Propolis+Pb	Pb	p
Glucose (mg/dl)	238.13±11.82	244.86±4.40	227.57±4.95	239.33±6.72	NS
Albumin (g/dl)	1.66±0.09	1.53±0.18	1.54±0.07	1.62±0.10	NS
Total cholesterol (mg/dl)	116.63±5.77	109.14±6.61	115.57±6.49	108.33±5.14	NS
Triglyceride (mg/dl)	53.50±4.34^{b}	63.57±2.28^{ab}	60.14±7.55^{b}	86.83±15.85^{a}	p<0.05
SGOT (IU/L)	90.71±16.66^{b}	110.43±8.86^{ab}	128.83±8.87^{a}	135.16±32.17^{a}	p<0.05
SGPT (IU/L)	13.83±1.28	13.50±2.95	13.20±3.76	13.66±1.36	NS
HDL (mg/dl)	66.89±4.41	73.10±13.40	65.28±4.42	69.42±8.51	NS
LDL (mg/dl)	25.56±2.86	34.62±6.56	34.97±3.65	38.03±2.31	NS

Results are expressed as mean±standard deviation. NS: Non significant.
[a-b] Mean values within a row with no common superscript differ significantly ($p<0.05$).

Table 4. MDA levels in plasma (nmol/ml) and some tissue (nmol/mg protein) of research groups (n = 6)

	Control	Vitamin C+Pb	Propolis+Pb	Pb	p
Plasma	4.40±0.49^{b}	5.06±0.82^{b}	5.33±0.68^{b}	7.70±0.85^{a}	p<0.01
Liver	0.88±0.03^{b}	1.15±0.15^{ab}	1.17±0.07^{ab}	1.53±0.18^{a}	p<0.05
Heart	0.61±0.08	0.72±0.08	0.80±0.09	0.97±0.2	NS

Results are expressed as mean±standard deviation. NS: Non significant.
[a-b] Mean values within a row with no common superscript differ significantly ($p<0.05$) ($p<0.01$).

be significantly higher in comparison to others ($p<0.05$).

DISCUSSION

Antioxidant matters added to the diet in order to support the protective activity of the antioxidant defense systems have a vital role. In the current study, the decreased trigliserid and SGOT levels were acceptable indicators that shown the addition of vitamin C (500 mg/kg diet) and propolis (1 g/kg diet) relieved the adverse effect of oxidative stress originating from lead (Zaidi et al., 2005; Yousef et al., 2006). In a study related to propolis supplementation conducted in broilers to determine the effects of propolis on biochemical parameters (Biavatti et al., 2003), it was determined that there is no effect on glucose, urea, creatin, cholesterol, trigliceride, SGOT, and SGPT. However, it was reported that propolis increased the blood lipid levels, cholesterol levels, and blood viscosity, and decreased the arteriosklerose (Akgul et al., 1997; Burdock, 1998; Stefano and Francesco, 2002).

In the current study, it was observed that propolis significantly decreased the triglyceride levels of the lead added group compare to the others ($p<0.05$). This finding was in agreement with Biavatti et al. (2003) who reported that propolis supported liver metabolism under oxidative stress. In a previous study using rats under oxidative stress, it was reported that SGOT levels increased under stress conditions (Zaidi et al., 2005). Similarly, in the current study, it was observed that the addition of 200 mg/kg of lead to the broiler diet significantly increased the plasma level of SGOT compare to the control group ($p<0.05$) (Zaidi et al., 2005); but this increase was not statistically significant in comparison to the others (Table 3). According to this finding, the decrease in triglyceride level of propolis group might be evidence that suggests that the addition of propolis relieved the adverse effects on triglyceride level of oxidative stress (Yousef et al., 2006).

When the MDA levels of the plasma and tissues were examined (Table 4), it was observed that the highest MDA level was in the lead added group in accordance with the increase of lipid peroxidation. On the other hand, the MDA levels of both the vitamin C and propolis added groups were lower compared to the lead added group. This decrease was particularly clear in the plasma ($p<0.01$). The MDA level of heart tissue was not affected by administrations. It is clear that vitamin C has an important effect on the absorption and excretion of lead but particularly in cases of high lead exposure (Hsu et al., 1998; Patra et al., 2001; Erdogan et al., 2004). According to the findings of the present study, it was shown that vitamin C had a positive effect on oxidative stress and decreased MDA levels in both plasma and tissue in accordance with other research. Propolis is a beekeeping product, which has antioxidant properties. This feature is caused by the main components, such as flavonoids and CAPE, in its structure. Flavonoids in the cell membrane protect the unsaturated fatty acids against oxidants as ascorbate (Havsteen, 2002). It was reported that CAPE decreased MDA levels by blocking ROS production as an antioxidant (Hosnuter et al., 2004). In a previous study where ascorbic acid was used to remove lead poisoning in broilers (Erdogan et al., 2004), it was reported that ascorbic acid addition at 100 mg/kg diet significantly decreased the MDA level ($p<0.01$). In a previous study related to propolis supplementation conducted to determine the treatment effect of propolis in cancer created female rats (Padmavathi et al., 2006), it was determined that porpolis addition significantly decreased the MDA levels as lipid peroxidation indicators in the breast and liver tissues of cancer created rats ($p<0.001$).

As shown in previous studies (Ates et al., 2006; Tatli Seven et al., 2009), MDA levels increased when the lipid peroxidation was formed. Similarly, the decreased MDA level in plasma or tissues indicated the decreased lipid peroxidation. According to the current study, it was thought that propolis addition was effective in decreasing the adverse effect of lipid peroxidation and would show similar antioxidant effects as vitamin C. Moreover, in a previous study where the antioxidant potential of propolis, vitamin E, and vitamin C were compared, propolis was found to be more effective than the other two (Okonenko et al., 2006).

Antioxidants inhibit lipid peroxidation by blocking the peroxidation chain reaction or by collecting reactive oxygen species. Major natural antioxidant enzymes in the body that are part of the cellular defense system are SOD, CAT, and GSH-Px. According to the studies, there is no accordance related to antioxidant enzyme levels in the case of lipid peroxidation (Okutan et al., 2005). Some studies (Wohaieb and Godin, 1987; Ozkaya et al., 2002) have reported a decrease while others have reported an increase in SOD and CAT activity in the case of lipid peroxidation (Huang et al., 1999; Aliciguzel et al., 2003).

When CAT activity in the current study was examined (Table 5), it was determined that lipid peroxidation increased the CAT activities of erythrocyte, liver, and heart tissues; the addition of vitamin C and propolis significantly decreased the CAT activity of heart tissue ($p<0.01$); the decrease of erythrocyte and liver CAT activity was not statistically changed. Similarly, the activity of SOD and GSH was in accordance with CAT activity. When the SOD and GSH activity increased (except plasma and heart tissue), in lead administrated group, these activities decreased by the addition of vitamin C and propolis (Tables 5 and 6).

The increase in CAT activity is a response to increased oxidative stress, which is formed because of a chronic increase in lipid peroxidation (Asayama et al., 1989; Yıldırım and Buyukbingol, 2003; Okutan et al., 2005). CAT

Table 5. Activities CAT in hemolysate (k/hHb) and some tissue (k/g protein) and SOD (U/ml) in blood of research groups (n = 6)

	Control	Vitamin C+Pb	Propolis+Pb	Pb	p
SOD	1.24±0.18^{b}	1.80±0.11^{b}	1.98±0.25^{b}	4.42±0.90^{a}	p<0.01
Hemolysate	1.72±0.46^{b}	3.82±0.62^{a}	4.42±0.74^{a}	5.35±0.62^{a}	p<0.01
Liver	0.68±0.06^{b}	0.88±0.05^{a}	0.89±0.06^{a}	0.97±0.08^{a}	p<0.05
Heart	3.51±0.22^{c}	5.22±0.22^{b}	5.62±0.60^{b}	8.40±0.44^{a}	p<0.01

Results are expressed as mean±standard deviation.
$^{a-c}$ Mean values within a row with no common superscript differ significantly (p<0.05) (p<0.01).

Table 6. GSH activities in blood (μmol/ml) and some tissues (nmol/ml) of research groups (n = 6)

	Control	Vitamin C+Pb	Propolis+Pb	Pb	p
Plasma	2.30±0.47^{b}	3.95±0.67^{ab}	4.27±0.47^{ab}	6.20±1.05^{a}	p<0.01
Liver	11.39±0.73	11.80±1.61	9.68±1.02	12.10±1.44	NS
Heart	9.84±0.75^{b}	18.41±2.59^{a}	18.27±3.92^{a}	20.29±2.30^{a}	p<0.01

Results are expressed as mean±standard deviation. NS: Non significant.
$^{a-b}$ Mean values within a row with no common superscript differ significantly (p<0.01).

activity in the tissues is quite variable. Liver, kidney, and erythrocyte had high activity while the brain, heart, lung, and ligaments had relatively low activity (Aebi, 1984).

In a previous study (Tatli Seven et al., 2009) where vitamin C (250 mg/kg diet) and different levels of propolis (0.5, 1, and 3 g/kg diet) were used antioxidant matter in broilers under heat stress (34°C), it was determined that the CAT activities of erythrocyte, liver, kidney, and heart significantly increased in chickens exposed to heat stress compared to the control group. But the addition of propolis at 3 g/kg diet significantly decreased this MDA level (p<0.01). On the other hand, it was reported that the brain and plasma SOD activities of rats under oxidative stress (Ichikawa et al., 2002), were decreased by adding 2% propolis to the diet.

In the current study, the plasma SOD activities of the control group, the vitamin C+lead, and the propolis+lead groups were significantly lower than the lead administrated group (p<0.01) (Table 5). Patra et al. (2001) reported that the addition of vitamin C significantly increased the CAT activities of liver and brain in rats exposed to lead compared to those of the control group. On the other hand, in a study where the effects of CAPE were investigated on antioxidant enzyme activity in rats exposed to cold stress (Ates et al., 2006), different results were obtained. It was determined that cold stress significantly decreased SOD, CAT, GPx, and GSH activities in the liver, but enzyme levels were significantly increased by adding CAPE (p<0.05).

In previous studies, where CAPE and tocopherol were used as antioxidant matter in rats exposed to oxidative stress, it was reported that there is no definite correlation between blood and tissues. Moreover, antioxidant effectiveness can vary depend on tissues and may not be seen similar always (Ichikawa et al., 2002; Irmak et al., 2003; Okutan et al., 2005). Therefore, the difference between research findings can be connected to the factors causing oxidative stress, the duration of exposure and breed of birds (Altan et al., 2003; Ates et al., 2006). Altan et al. (2003) also reported that antioxidant enzyme levels were significantly increased in *Ross-breed* broilers under heat stress compared to the control group but similar increases were only observed in the GPx activity of *Cobb-breed* broilers while the other antioxidant enzyme levels were not different (p>0.05).

CONCLUSIONS

As a result, the activities of SOD, CAT, and GSH increased depending on the cellular defense mechanisms under oxidative stress in accordance with the increased MDA level. Vitamin C (500 mg/kg diet) and propolis (1 g/kg diet) decreased the SOD activity and have shown a tendency to reduce CAT and GSH levels. Propolis used as an antioxidant in broilers exposed to lead showed similar antioxidant effects as vitamin C in the case of oxidative stress. Using 1 g/kg of propolis supplementation in maize-soybean meal type broiler diets may relieve the adverse effect of oxidative stress on the antioxidant defense system.

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