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Oxidative Stress Biomarkers and Biochemical Profile in Broilers Chicken Fed Zinc Bacitracin and Ascorbic Acid under Hot Climate

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ABSTRACT

Dietary manipulation of heat induced oxidative stress is one of the most widely used method to alleviate the negative effect of heat stress. The combination of two or more potential antioxidants are known to protect against heat stress. Therefore, 200 day old broiler chicks were raised under hot climate to determine the effect of ascorbic acid and zinc bacitracin and their combination as antioxidants. Birds were divided into four treatments; the first treatment (T_1), the control was provided with basal diet. Second (T_2) and third (T_3) treatments were provided with 1 g ascorbic acid and 100 mg zinc bacitracin per kg basal diet, respectively. The fourth treatment (T_4) was provided with a combination of T_2 and T_3 for 42 days. Performance parameters were reported every fortnight. Blood samples were collected every fortnight and the harvested sera were used to determine oxidative stress biomarkers. Ascorbic acid and zinc bacitracin were safe for heat stressed birds since the liver and kidney function indicators were not changed. Ascorbic acid and zinc bacitracin lowered lipid peroxidation level (Malondialdehyde; MDA) and increased the activity of catalase (CAT), glutathione-S-transferase (GST) and Super Oxide Dismutase (SOD) whereas, glutathione peroxidase (GPx) activity remained unchanged in all treatments. Serum ascorbic acid concentration was significantly ($p<0.05$) increased accompanied with hypocholesterolemia in heat stressed broilers receiving ascorbic acid compared to the control. Performance parameters were not influenced by feed additives. In conclusion, ascorbic acid was more potent than zinc bacitracin and their combination in increasing the level of enzymatic and non enzymatic antioxidants.

Key words: Heat stress, antioxidants, poultry, biochemistry, performance

INTRODUCTION

The detrimental effects of high ambient temperature on broiler performance have been widely documented (Ramnath *et al.*, 2008; Rashidi *et al.*, 2010; Ali *et al.*, 2010). Feed consumption and growth rate decrease at high ambient temperature (El-Habbak *et al.*, 2011). Antioxidant status of the organism changed as result of heat induced oxidative stress (Sahin *et al.*, 2001). Oxidative stress is caused by free radicals, reactive oxygen species which damage DNA, biomembrane lipids, proteins and other macromolecules (Lu *et al.*, 2010). The primary source of reactive oxygen species is leakage of electron from the respiratory chain during the reduction of molecular oxygen to water generating superoxide anion in chicken (Mujahid *et al*., 2005; Lu *et al*., 2010). However, free radicals can be scavenged by the use of antioxidant system including non enzymatic components and a series of antioxidant enzymes (Lu *et al*., 2010). Non enzymatic components include glutathione, selenium, Vitamin C and E. The antioxidant enzymes include glutathione peroxidase, catalase and superoxide dismutase which are the most major antioxidant enzymes that are capable to minimize oxidative stress in the organelles (Cadenas and Davies, 2000). The degree of lipid peroxidation is often used as an indicator of ROS mediated damage (Kunn and Borchert, 2002) and the concentration of MDA in blood and tissues are generally used as biomarkers of lipid peroxidation (Sehirli *et al*., 2008; Yousef *et al*., 2009). Alleviation of the negative effect of high environmental temperature through cooling of animal house is impractical and costly, therefore workers devoted to dietary manipulation. Uses of Vitamin C (ascorbic acid) as feed additive was aimed at reducing the heat stress in birds (Sahin *et al*., 2002; Asli *et al*., 2007). Ascorbic acid also referred to as ascorbate or Vitamin C participates in numerous biochemical reactions (Seyrek *et al*., 2004). Under heat condition birds are not able to synthesize sufficient amount of ascorbic acid (Kutlu and Forbes, 1993) and supplemented ascorbic acid could significantly reduce the body temperature (Orban *et al*., 1993). Zinc bacitracin (100 mg kg^{-1} diet) decreased the adverse effect of exposing hen to high temperature (Manner and Wang, 1991), an effective growth promoter with little tendency to produce bacterial resistance and is not absorbed from the gut and thereby of low residues in chicken meat (King, 1970). In addition, Zinc bacitracin reduced the number of coliform bacteria in broiler ileum and increased pancreatic amylase and lipase activities (Engberg *et al*., 2000). The combination of two or more potential antioxidants may help each to more effectively neutralize the systemic free radicals antioxidants are known to protect against heat stress. The combined effect of ascorbic acid and zinc bacitracin was not investigated therefore, the present study aimed to reveal their effects as reflected on lipid peroxidation and oxidative stress biomarkers.

MATERIALS AND METHODS

Birds, feed and additives: A total of 200 day old broiler chicks (Ross 308; 40±3 g body weight) were assigned to four treatments and housed in an open sided poultry house at the Research Station, King Faisal University, Al-Ahsa, Saudi Arabia. The pens provided a floor area of 1.5 m^2. Each treatment constitutes 5 replicates (10 birds $replicate^{-1}$). The ambient temperature and relative humidity were monitored daily (39°C±2).

Commercial broiler feed (ARASCO, Saudi Arabia; starter code No. 24203; Finisher code No. 24402) was used as basal diet (untreated heat stressed birds, Control; T_1) throughout the experimental period (day 1-42). Ascorbic acid (1 g kg^{-1} basal diet; T_2), zinc bacitracin (100 mg kg^{-1} basal diet; T_3) and their combination (T_2 and T_1; T_4) were provided to heat stressed birds. Feed was formulated from cereal, soybean meal, vegetable oil, vitamins and bioplex minerals. Coccidiostat (Avatec/Cygro) was added only in starter diet. The calculated analysis of the commercial diet is shown in Table 1.

Performance parameters: Feed intake and body weight gain were recorded on day 14, 28 and 42. Dead birds were removed and reported daily. At 42 days of age, three birds per replicate were randomly chosen, slaughtered and their liver, heart and spleen were collected, weighed and calculated as a percentage of live body weight. The experiment complied with the guidelines of King Faisal University with respecting to birds experimentation and care of birds under study.

Table 1: Calculated analysis of basal diet

Diet ingredients (g kg^{-1})	Starter	Finisher
Metabolizable energy (kcal kg^{-1})	2900	3000
Minimum crude protein (%)	21.0	18.5
Minimum crude fat (%)	2.5	3.0
Maximum fibre (%)	3.0	3.5
Calcium (%)	1.0	0.9
Available phosphorus (%)	0.42	0.4
Sodium (%)	0.15	0.15
Lysin (%)	1.20	1.0
Methionine (%)	0.50	0.45
Methionine+cystine (%)	0.85	0.80
Vitamin A (IU kg^{-1})	12000	12000
Vitamin D (IU kg^{-1})	3000	3000
Vitamin E (mg kg^{-1})	60	60
Vitamin C (mg kg^{-1})	100	100
Vitamin K (mg kg^{-1})	4.0	4.0
Vitamin B_1 (mg kg^{-1})	3.0	3.0
Vitamin B_2 (mg kg^{-1})	8.0	8.0
Vitamin B_6 (mg kg^{-1})	5.0	5.0
Vitamin B_{12} (mg kg^{-1})	0.03	0.03
Niacin (mg kg^{-1})	40.0	40.0
Pantothenic acid (mg kg^{-1})	15	15
Folic acid (mg kg^{-1})	2.0	2.0
Biotin (mg kg^{-1})	0.2	0.2
Choline added (mg kg^{-1})	900	900
Cobalt (mg kg^{-1})	0.5	0.5
Copper (mg kg^{-1})	8.0	8.0
Iodine (mg kg^{-1})	2.0	2.0
Iron (mg kg^{-1})	35.0	35.0
Manganese (mg kg^{-1})	90	90
Selenium (mg kg^{-1})	0.2	0.2
Zinc (mg kg^{-1})	70	70
Antioxidant (mg kg^{-1})	125	125

Blood sampling and biochemical analysis: Blood samples were obtained from the wing vein of five birds of each treatment every fortnight (three times throughout the experimental period) using a 3 mL syringe and 23 gauge needle, harvested sera were used for determination of serum biochemical parameters. Serum Catalase (CAT) activity was assessed by measuring catalase degradation of H_2O_2 using a redox dye (ELISA Kit: QuantiChrom^{TM}, BioAssay Systems, USA, Catalog No. ECAT-100) according to Cowell *et al.* (1994). SOD activity was measured by the xanthine oxidase method (ELISA Kit: Cayman Chemical Company, USA, Catalog No. 706002), which monitors the inhibition of nitro blue tetrazolium reduction by the sample (Sun *et al.*, 1988). Serum glutathione peroxidase (GPx) activity was assessed by using H_2O_2 and an electron donor dye that forms a pink color during the peroxide reaction (ELISA Kit: QuantiChrom^{TM}, BioAssay Systems, USA, Catalog No. DPOD-100) according to Kokkinakis and Brooks (1979). Glutathione-S- transferase activity was assessed by measuring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione (ELISA Kit: Cayman Chemical Company, USA, Catalog No. 703302) according to Habig *et al.* (1974). Serum lipid peroxidation level was assessed by determining thiobarbituric acid reactive substances (TBARS) produced during peroxidation (ELISA Kit: QuantiChrom™, BioAssay Systems, USA, Catalog No. DTBA-100) following Ohkawa *et al.* (1979). Serum ascorbic acid concentration was assessed by its oxidation with ascorbate oxidase resulting in the production of H_2O_2 which react with a specific dye to form a pink colored product (ELISA Kit: EnzyChrom™, BioAssay Systems, USA, Catalog No. EASC-100) according to Baker and Lowe (1985). The sera were also used for spectrophotometric determination of the activities of Aspartate Transaminase (AST) and Alanine Transaminase (ALT) as directed by Reitman and Frankel (1957). In addition, serum glucose, total protein, albumin and globulin values were determined spectrophotometrically as implied by the methods of Trinder (1969), Doumas *et al.* (1981), Reinhold (1953) and Coles (1974), respectively. Serum blood urea nitrogen, uric acid and creatinine were determined according to the method described by Tabacco *et al.* (1979) and Todd and Henry (1984), respectively. Furthermore, the obtained sera were used for spectrophotometric analysis of serum triacylglycerol (TAG), total cholesterol and HDL-c by using of enzymatic method of commercial kits according to the methods of Gottfried and Rosenberg (1973), Zak *et al.* (1954) and Lopes-Virella *et al.* (1977), respectively. Very low density lipoprotein cholesterol (VLDL-c) was calculated by division of TAG by 5 While the LDL-c was calculated as total cholesterol-(HDL-c+VLDL-c) = mg dL^{-1} (Bauer, 1982).

Statistical analysis: All data was presented as Mean±SE of mean by using student t-test. All tests were performed using computer package of the statistical analysis system (SAS, 2002).

RESULTS

The present findings indicated that, both feed additives, Ascorbic acid and zinc bacitracin were safe for heat stressed birds as reflected on unchanged liver and kidney function. Heat stress elevated the level of lipid peroxidation as reflected on high values of Malondialdehyde (MDA), in addition, heat stress also depleted the antioxidant capacity of the birds as reflected on decreased catalase (CAT), glutathione-S-transferase (GST) and Super Oxide Dismutase (SOD) activities. In the contrary, Ascorbic acid and zinc bacitracin lowered lipid peroxidation level and increased the activity of antioxidant enzymes. Hypocholesterolemia and high ascorbic acid level were observed in heat stressed broilers receiving ascorbic acid compared to the control. Feed intake, feed conversion ratio and the relative weight of the heart, liver and spleen were the same in both heat stressed and treated birds. The present finding indicated also that, ascorbic acid was more potent than zinc bacitracin and their combination in counteracting heat stress enzymatically and nonenzymatically.

Performance parameters: The results shown in Table 2 revealed that the combination of ascorbic acid and zinc bacitracin significantly ($p<0.05$) promoted the growth of broiler under heat stress in the first two weeks. Zinc bacitracin supplementation significantly ($p<0.05$) reduced the mortality rate (2%) compared to the control; however, the average mortality rate in the other group was 4%. A significant ($p<0.05$) growth promoting effect was only observed in heat stressed birds fed combination of ascorbic acid and zinc bacitracin during the first two weeks of the experiment compared to the control and other treated groups. Neither zinc bacitracin nor ascorbic acid had a significant influence on feed intake and feed conversion ratio of the experimental heat stressed birds (Table 2). Table 3 demonstrated that, relative weights of heart, liver and spleen were not significantly ($p>0.05$) affected by dietary treatments compared to the control.

Table 2: Performance characteristics of heat stressed broiler chicken supplemented with ascorbic acid and/or zinc bacitracin during 2nd, 4th and 6th weeks

Time	Parameters	T_1	T_2	T_3	T_4
1-14 days	Feed intake (g $bird^{-1}$)	370±4.90	380±3.7	371±5.7	382±5.9
	Body weight gain (g $bird^{-1}$)	175±7.40	185±5.0	178±7.5	200±5.3*
	Feed conversion ratio	2.1±0.01	2.1±0.1	2.1±0.1	1.9±0.1
	Mortality (%)	2	0	0	2
14-28 days	Feed intake (g $bird^{-1}$)	515±4.0	518±3.1	516±4.1	520±2.9
	Body weight gain (g $bird^{-1}$)	273±5.2	272±5.4	283±5.0	278±8.8
	Feed conversion ratio	1.9±0.2	1.9±0.1	1.8±0.1	1.9±0.1
	Mortality (%)	2	4	0	2
28-42 days	Feed intake (g $bird^{-1}$)	566±8.3	558±8.6	560±8.1	551±9.1
	Body weight gain (g $bird^{-1}$)	289±5.6	290±5.0	301±9.0	285±9.8
	Feed conversion ratio	2±0.1	1.9±0.1	1.9±0.3	1.9±0.2
	Mortality (%)	6	0	6	6

T_1: Control group, T_2: Ascorbic acid treated group, T_3: Zinc bacitracin treated group, T_4: Ascorbic acid and bacitracin combination treated group, Values are Mean±SD, *Statistically significant when compared to control (treatment 1) at $p<0.05$

Table 3: Relative weight (organ weight/live weight×100) of some internal organs of heat stressed broiler chicken supplemented with ascorbic acid and/or zinc bacitracin at day 42

Parameters	T_1	T_2	T_3	T_4
Liver	2.02±0.33	2.14±0.18	2.21±0.37	2.17±0.41
Heart	0.34±0.05	0.35±0.07	0.33±0.04	0.38±0.08
Spleen	0.08±0.03	0.09±0.04	0.09±0.03	0.08±0.05

T_1: Control group; untreated heat stressed birds, T_2: Heat stressed birds treated with Ascorbic acid, T_3: Heat stressed birds treated with Zinc bacitracin, T_4: Heat stressed birds treated with combination of ascorbic acid and bacitracin, Values are Mean±SD

Biochemical analysis: The present study reported insignificant ($p>0.05$) difference in serum values of glucose, total protein, albumin, globulin, TAG, VLDL-c, total cholesterol, AST, ALT, creatinine, urea and uric acid values in all heat stressed birds throughout the experimental period (Table 4). However, ascorbic acid value was significantly ($p<0.05$) higher only in serum of heat stressed birds fed ascorbic acid during 2nd, 4th and 6th weeks of the experiment (60.4±1.2; 58.8±0.9; 62.0±0.7 μM) compared to untreated heat stressed birds (23.6±1.1; 23.9±0.5; 23.5±3.1 μM), respectively (Table 4). In addition, HDL-c value was significantly ($p<0.05$) higher (1.79±0.02 mmol L^{-1}) and LDL-c value was significantly ($p<0.05$) lower (1.06±0.01 mmol L^{-1}) in heat stressed birds fed ascorbic acid only during the first two weeks of the experiment compared to the untreated heat stressed birds (1.51±0.05 and 1.40±0.06 mmol L^{-1}), respectively (Table 4).

Results summarized in Table 5 demonstrated that, the activity of Catalase enzyme was significantly ($p<0.05$) higher only in heat stressed birds treated with ascorbic acid during week 3 and 4 of the experiment (104.4±2.32 IU L^{-1}), when compared with untreated heat stressed birds (83.5±2.22 IU L^{-1}). The other heat stressed treated birds remained comparable ($p>0.05$) to the untreated heat stressed birds throughout the experimental period. Moreover, the activity of serum GST was significantly ($p<0.05$) higher in heat stressed birds treated either with ascorbic acid (1.6±0.16 nmol min mL^{-1}) or zinc bacitracin only (1.9±0.14 nmol min mL^{-1}) during the last two weeks (Week 5 and 6) of the experiment when compared with heat stressed untreated birds (0.9±0.14 nmol min mL^{-1}; Table 5). The present findings also indicated that, the activity of SOD was significantly ($p<0.05$) higher in heat stressed birds treated with ascorbic acid during

Table 4: Biochemical parameters of heat stressed broiler chicken supplemented with ascorbic acid and/or zinc bacitracin for 6 weeks

Parameters	Time (week)	T_1	T_2	T_3	T_4
Ascorbic acid (μM)	2nd	23.60±1.1	60.40±1.2*	24.30±1.4	24.50±1.2
	4th	23.90±0.5	58.80±0.9*	22.70±1.3	22.40±1.2
	6th	23.50±3.1	62.00±0.7*	19.80±2.0	21.90±1.0
Glucose (mmol L^{-1})	2nd	4.05±0.06	3.97±0.04	4.120±0.04	4.15±0.06
	4th	4.27±0.08	4.41±0.03	4.42±0.02	4.32±0.02
	6th	4.05±0.09	4.05±0.02	3.96±0.06	3.88±0.04
Total protein (g L^{-1})	2nd	33.50±2.2	35.40±1.1	33.30±1.2	34.20±2.2
	4th	32.10±1.1	32.20±1.1	31.90±1.1	33.20±1.1
	6th	36.30±1.2	37.10±1.1	39.10±1.1	36.20±2.2
Albumin (g L^{-1})	2nd	14.50±1.2	14.40±1.1	15.30±1.3	15.20±1.4
	4th	15.10±1.2	15.20±1.2	13.90±1.1	14.20±1.2
	6th	15.30±1.1	14.10±1.3	15.10±1.2	15.20±1.3
Globulin (g L^{-1})	2nd	19.00±2.2	21.00±1.3	18.00±2.2	19.00±2.2
	4th	17.00±1.2	18.00±1.1	18.00±1.1	18.00±1.1
	6th	21.00±1.1	23.00±1.2	24.00±2.2	21.00±1.2
Triglyceride (mmol L^{-1})	2nd	0.80±0.02	0.80±0.02	0.85±0.05	0.81±0.05
	4th	0.94±0.03	0.91±0.02	0.97±0.08	0.91±0.03
	6th	0.80±0.04	0.78±0.01	0.79±0.01	0.81±0.01
Cholesterol (mmol L^{-1})	2nd	3.07±0.08	3.01±0.03	3.09±0.06	3.07±0.05
	4th	2.91±0.09	2.85±0.01	2.84±0.01	2.76±0.06
	6th	2.98±0.05	2.90±0.04	2.98±0.04	3.01±0.03
HDL-c (mmol L^{-1})	2nd	1.51±0.05	1.79±0.02*	1.58±0.03	1.53±0.03
	4th	1.47±0.06	1.53±0.01	1.54±0.01	1.42±0.06
	6th	1.46±0.05	1.48±0.06	1.53±0.03	1.52±0.02
LDL-c (mmol L^{-1})	2nd	1.40±0.06	1.06±0.01*	1.34±0.04	1.38±0.02
	4th	1.25±0.06	1.14±0.05	1.11±0.06	1.16±0.03
	6th	1.36±0.05	1.26±0.06	1.29±0.05	1.33±0.04
VLDL-c (mmol L^{-1})	2nd	0.16±0.02	0.16±0.03	0.17±0.06	0.16±0.04
	4th	0.19±0.05	0.18±0.01	0.19±0.04	0.18±0.05
	6th	0.16±0.03	0.16±0.03	0.16±0.02	0.16±0.03
ALT (IU L^{-1})	2nd	16.90±2.6	19.10±2.0	19.30±2.1	17.70±2.9
	4th	9.90±1.2	9.00±1.9	8.40±1.4	7.10±1.9
	6th	10.70±1.4	11.50±1.6	11.80±0.9	10.90±1.3
AST (IU L^{-1})	2nd	84.30±1.6	83.80±1.0	81.40±1.1	85.70±0.9
	4th	86.70±1.2	86.60±0.9	85.70±1.0	85.70±1.1
	6th	96.60±1.4	97.10±1.6	98.50±0.9	97.60± 1.3
Creatinine (μmol L^{-1})	2nd	88.40±2.6	89.28±2.9	91.05±1.6	90.17±1.4
	4th	91.05±1.4	89.28±1.3	89.28±1.4	90.17±1.1
	6th	90.17±1.5	91.94±1.1	90.17±1.3	91.05±1.4
Uric acid (μmol L^{-1})	2nd	362.83±5.6	368.78±2.9	374.72±4.6	362.83±3.4
	4th	344.98±2.4	341.98±2.3	338.09±2.4	339.04±2.1
	6th	380.07±0.5	386.64±4.1	390.46±2.3	392.57±1.4
Urea (μmol L^{-1})	2nd	0.85±0.03	0.82±0.04	0.79±0.06	0.81±0.02
	4th	0.74±0.01	0.75±0.2	0.71±0.04	0.69±0.07
	6th	0.79±0.05	0.79±0.5	0.75±0.04	0.77±0.02

T_1: Control group; untreated heat stressed birds, T_2: Heat stressed birds treated with ascorbic acid, T_3: Heat stressed birds treated with Zinc bacitracin, T_4: Heat stressed birds treated with combination of ascorbic acid and bacitracin, Values are Mean±SD, *Statistically significant when compared to control (treatment 1) at $p<0.05$

Table 5: Oxidative stress biomarkers of heat stressed broiler chicken supplemented with Ascorbic acid and/or zinc bacitracin for 6 weeks

Parameters	Time (week)	T_1	T_2	T_3	T_4
Catalase (IU L^{-1})	2nd	69.6±1.83	70.8±1.41	70.8±1.10	68.9±1.61
	4th	83.5±2.22	104.4±2.32*	84.8±2.63	82.9±2.4
	6th	68.6±1.21	69.8±2.81	67.2±1.41	66.7±2.22
G-S-T (nmol min mL^{-1})	2nd	1.1±1.11	1.0±0.12	1.1±0.11	1.3±0.13
	4th	1.2±0.11	1.2±0.13	1.3±0.10	1.3±0.11
	6th	0.9±0.14	1.6±0.16*	1.9±0.14*	0.9±0.12
SOD (IU mL^{-1})	2nd	6.0±0.54	5.9±0.90	7.4±0.24*	7.7±0.29*
	4th	4.9±0.28	8.2±0.51*	7.3±0.51*	6.8±1.03*
	6th	5.2±1.01	5.5±0.60	6.3±0.70*	7.6±0.70*
GPx (IU L^{-1})	2nd	127.1±2.91	130.1±2.01	128.3±1.11	131.3±1.53
	4th	100.2±1.62	102.0±1.10	101.1±1.30	98.5±2.72
	6th	100.7±1.53	101.1±1.70	99.7±1.00	98.8±1.31
MDA (μM)	2nd	10.3±1.1	11.1±1.12	9.5±1.52	10.0±1.21
	4th	9.8±1.4	9.3±1.80	8.9±1.12	10.3±1.31
	6th	8.6±0.11	8.1±0.01*	8.0±0.02*	8.0±0.0 3*

T_1: Control group; untreated heat stressed birds, T_2: Heat stressed birds treated with ascorbic acid), T_3: Heat stressed birds treated with Zinc bacitracin, T_4: Heat stressed birds treated with combination of ascorbic acid and bacitracin, Values are Mean±SD, *Statistically significant when compared to control (treatment 1) at $p<0.05$

week 3 and 4 of the experiment (8.2±0.51 IU mL^{-1}) when compared with heat stressed untreated birds (4.9±0.28 IU mL^{-1}; Table 5). The activity of this enzyme was significantly ($p<0.05$) higher in heat stressed birds treated with zinc bacitracin alone (7.4±0.24; 7.3±0.51; 6.3±0.70 IU mL^{-1}) or in combination with ascorbic acid (7.7±0.29; 6.8±1.03; 7.6±0.70 IU mL^{-1}) during 2nd, 4th and 6th weeks of the experiment when compared with heat stressed untreated birds (6.0±0.54; 4.9±0.28; 5.2±1.01 IU mL^{-1}), respectively (Table 5). Furthermore, the activities of GPx in heat stressed birds treated with ascorbic acid and zinc bacitracin either alone or in combination remained unchanged ($p>0.05$) when compared with heat stressed untreated birds (Table 5). The concentration of MDA was significantly ($p<0.05$) lower in birds fed ascorbic acid (8.1±0.01 μM) and zinc bacitracin (8.0±0.02 μM) either alone or in combination (8.0±0.03 μM) when compared with the control (8.6±0.11 μM) during the last two weeks of the experiment (Table 5).

DISCUSSION

The increase in the body weight gain observed during the second week in the birds receiving ascorbic acid and zinc bacitracin combination was supported by the finding of (Lohakare *et al*., 2005; El-Habbak *et al*., 2011; Talebi and Khademi, 2011) who reported an improvement in broiler chicks fed Vitamin C and also the improvement of body weight in chicks fed zinc bacitracin (Waldroup *et al*., 1986; Mujdat *et al*., 1999; Abdulrahim *et al*., 1999), since there is scarcity or in availability of literature concerned with combination of ascorbic acid and zinc bacitracin and our finding stand first as a report concerned with the use of ascorbic acid and zinc bacitracin combination in broiler feed. The significant ($p<0.05$) decrease in mortality observed in zinc bacitracin treated birds comes in accordance with the findings of Pinheiro *et al*. (2004) in rabbits and on the contrary with Damron *et al*. (1991) who did not find any effect of zinc bacitracin on mortality of broiler breeder. The data concerning the effect of ascorbic acid and zinc bacitracin in broiler chicken was inconsistent. Our findings demonstrated that ascorbic acid did not show any effect on feed intake, feed conversion ratio which is consistent with previous findings in broiler chicks (Lohakare *et al*., 2005; Erdogan *et al*., 2005) and Turkeys (Konca *et al*., 2008). On the other hand, other publications indicted that, dietary ascorbic acid alleviated the negative effect of heat stress on feed intake and feed conversion ratio in broiler chicks (El-Habbak *et al*., 2011; Talebi and Khademi, 2011), Layers (Khan and Sardar, 2005) and Japanese quail (Sahin *et al*., 2003). Manner and Wang (1991) reported that, feed efficiency was not affected by inclusion of 3.1% zinc bacitracin in heat stressed hens diet. However, Abdulrahim *et al*. (1999) reported that, feed conversion was reduced by zinc bacitracin alone but was improved by the use of *L. acidophilus* and bacitracin in combination in broiler chicken. Furthermore, feed utilization was improved when broiler chickens fed about half of the dose of the current study (Waldroup *et al*., 1986). However, feeding broilers zinc bacitracin significantly improved growth rate and feed conversion (Choi and Ryu, 1987). The unchanged relative weights of heart, liver and spleen observed in the present study come in accordance with those obtained by Fletcher and Cason (1991) and Lohakare *et al*. (2005) in commercial broiler and with those obtained by Konca *et al*. (2008) in Turkeys. However, Sahin *et al*. (2003) suggested that dietary Vitamin C increased liver, heart and spleen weights in broilers.

The insignificant difference in serum total protein, albumin, globulin, TAG, cholesterol, AST and ALT values which observed in all heat stressed treatments comes in accordance with the results obtained by Ali *et al*. (2010) in slow growing chicks subjected to heat stress. In addition, Erdogan *et al*. (2005) reported insignificant changes in albumin, total protein, ALT and AST in broiler chicken fed ascorbic acid as a trial to alleviate dietary lead exposure. However, El-Habbak *et al*. (2011) reported that heat stress significantly decreased TAG, AST, ALT and glucose in heat stressed broiler. Khan and Sardar (2005) reported significant increase in ALT and AST in Vitamin C treated layers. The unchanged ALT and AST observed in the present study give an indication that liver and kidney function were not disturbed when ascorbic acid and/or zinc bacitracin provided in the broiler chicken diet. Cholesterol is transported via blood by lipoproteins. HDL-c (good cholesterol) transports cholesterol from tissues to liver however LDL-c (bad cholesterol) transports it from liver to tissues. Therefore, an increase in serum HDL-c cholesterol is an indication of high rate of transportation of cholesterol from tissues to liver and subsequent transformation of cholesterol into bile acid by the aids of ascorbic acid which control the microsomal 7a-hydroxylation (the rate limiting enzyme in cholesterol catabolism). HDL-c/LDL-c ratio gives an indication as to whether cholesterol is likely to be deposited in the arteries or not (Genest *et al*., 1999). A ratio favoring HDL-c as noticed in the present study considered beneficial to human health protecting from coronary heart disease, vascular collapse and kidney disease when human consume such meat. Vahdatpour *et al*. (2009) reported a significant increase in cholesterol, TAG, HDL-c and LDL-c in broiler chicken received corticosterone as stress alternative hormone. The same authors demonstrated that, Vitamin C alleviated the adverse effect of heat stress in broiler chicks. The significant higher HDL-c and significant lower LDL-c levels in ascorbic acid treated birds come in consistence with results obtained by Seyrek *et al*. (2004) in Japanese quails. In contrast to our results, Ogboko (2011) reported that HDL-c was significantly decreased and LDL-c was significantly increased when zinc bacitracin was added to broiler diet either protein or plant based diet. Similar results demonstrated significantly higher values of ascorbic acid in blood of ascorbic acid treated layers (Khan and Sardar, 2005) and Japanese Quails (Sahin *et al*., 2003).

The oxidative stress can be identified as an imbalance between prooxidants and antioxidants with favor of the prooxidants the oxidative damage will be induced (Sies, 1991). Decreased antioxidant defenses or increase free radicals resulting from different stressors may be detrimental to the tissues and caused disease manifestation. At high environmental temperature the birds maintains optimal body temperature by increasing evaporative cooling which in turn increased their metabolism and energy consumption (Gomez *et al.*, 2002). The increased need for energy is met by mobilization of lipids from the stored fats. Higher level of MDA indicates peroxidation of lipids (Whittow, 1994). In the present study, feed consumption were comparable in all treatments, therefore, the source of extra energy was not obtained from feed and unfortunately the body depends on mobilization of fat with generation of free radicals and higher level of MDA as a result of lipid peroxidation. The lower levels of serum MDA in birds treated with ascorbic acid and zinc bacitracin either alone or in combination as compared to the control, suggested that ascorbic acid and/or zinc bacitracin ameliorates the negative effect of heat stress as antioxidants. The significant higher level of MDA in untreated heat stressed broilers which observed in the present study come in consistence with (El-Shaieb *et al.*, 2009) in broiler chicken, (Ramnath *et al.*, 2008) in egg type domestic chicken and with the findings obtained by Yardebi and Turkay (2008) and Puthpongsiriporn *et al.* (2001) in heat stressed laying hen. The significant reduction of MDA in ascorbic acid treated birds comes in accordance with (Erdogan *et al.*, 2005; El-Shaieb *et al.*, 2009) in broiler chicken.

The significant increase of CAT, GST and SOD activities in ascorbic acid treated birds and GST and SOD in zinc bacitracin treated birds and SOD in bird fed combination of ascorbic acid and zinc bacitracin observed in the current study may be considered as a protective mechanism against oxidative stress and lipid peroxidation (Davies, 1995; Altan *et al.*, 2003; Seven *et al.*, 2009). Similar results were demonstrated that Vitamin C supplementation increased the antioxidant defense and ameliorate the oxidative stress of heat stressed broiler (Seven, 2008) and white leghorn layers (Panda *et al.*, 2008). This might give an impression of the potentiality of ascorbic acid and zinc bacitracin for stimulation of antioxidants enzymes biosynthesis to ameliorate heat induced oxidative damage. However, further investigation at the molecular level is required in the future studies. The antioxidant effect of ascorbic acid and zinc bacitracin is underlined by the reduction of lipid peroxidation mentioned above. Mechanism that explained how Vitamin C alleviated the negative effect of heat stress was described (Sheila and Cheryl, 1978; Kutlu and Forbes, 1993; Sahin *et al.*, 2002; Panda *et al.*, 2008). They explained that, heat stress stimulates the biosynthesis and secretion of corticosteroids and this can be antagonized by dietary supplementation of ascorbic acid.

CONCLUSION

The current study demonstrated that, ascorbic acid alleviated the negative effect of heat stress by stimulating the biosynthesis and secretion of antioxidant enzymes which scavenging the free radicals as reflected by increasing the activities of CAT, SOD and GST together with reduced MDA level preventing cells from lipid peroxidation. Ascorbic acid was more potent than zinc bacitracin and their combination in increasing the level of enzymatic and non enzymatic antioxidants.

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