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Biochemical Markers of Oxidative Stress in Tissues of Broiler Chickens Fed Zinc Bacitracin and Ascorbic Acid under Hot Climate

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ABSTRACT

Previous study demonstrated that, Ascorbic Acid (AA) was more potent than Zinc Bacitracin (ZB) and their combination in increasing levels of serum enzymatic and non-enzymatic antioxidants of broiler chickens reared under hot climate. However, the levels of these enzymes in the tissues of these birds were not estimated earlier. Previously, 200 day old broiler chicks 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 AA and 100 mg ZB/kg basal diet, respectively. The fourth treatment (T_4) was provided with a combination of T_2 and T_3 for 42 days. At the end of the experiment, liver, heart and kidney tissues were collected and stored frozen until used in the current study for analysis of biochemical markers of oxidative stress. AA and ZB either alone or in combination significantly decreased the lipid peroxidation level (Malondialdehyde; MDA) and increased the activities of glutathione peroxidase (GPx) in all examined tissues compared to the control untreated heat stressed birds. Catalase (CAT), Glutathione-S Transferase (GST) and Glutathione Reductase (GR) activities were only increased in the liver of heat stressed birds treated with AA. However, GST and GR activities were also increased in heart of heat stressed birds treated with either AA or ZB compared to control. In conclusion, AA was more potent than ZB and their combination in increasing the level of enzymatic antioxidants in tissue of heat stressed birds.

Key words: Oxidative stress, heat, tissues antioxidants, broiler, biochemistry

INTRODUCTION

High ambient temperature decreased the broiler performance (Rashidi *et al*., 2010; Ali *et al*., 2010) particularly feed consumption and growth rate (El-Habbak *et al*., 2011). Antioxidant status of the organism is activated as response to heat induced oxidative stress (Sahin *et al*., 2001). The oxidative stress is caused by Reactive Oxygen Species (ROS) (Lu *et al*., 2010) which produced due to 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). The enzymatic or non-enzymatic antioxidant systems of the body are responsible for scavenging of these ROS protecting DNA and macromolecules from deterioration (Lu *et al*., 2010). Glutathione, selenium, Vitamin C and E are the main non enzymatic antioxidants whereas, GPx, CAT and SOD are the

major antioxidant enzymes (Cadenas and Davies, 2000). Level of MDA in blood and tissues were used as biomarkers of lipid peroxidation (Sehirli *et al*., 2008; Yousef *et al*., 2009; Ismail *et al*., 2013) and MDA level is directly proportional to degree of lipid peroxidation (Kuhn and Borchert, 2002; Ismail *et al*., 2013). The high costs and impractical applications of cooling of animal house are the main reasons of thinking about the possibility of using dietary manipulation to alleviate the detrimental effect of heat stress. Ascorbic acid was used as feed additive for reducing the heat stress in birds (Sahin *et al*., 2002; Asli *et al*., 2007). Birds are not able to synthesize sufficient amount of AA under heat stress condition (Kutlu and Forbes, 1993) and dietary supplementation of AA is essential to the elevated body temperature (Orban *et al*., 1993). The ZB (100 mg kg^{-1} diet) was able to ameliorate the adverse effect of heat stress (Manner and Wang, 1991). It acts as growth promoter of low bacterial resistance and of low residues in chicken meat because it is not absorbed from the gut (King, 1970). The combined effect of ascorbic acid and zinc bacitracin was investigated earlier (Ismail *et al*., 2013) and authors demonstrated that AA was more potent than ZB and their combination in increasing the level of enzymatic antioxidants in serum of heat stressed birds. However, the effect of AA and/or ZB on tissue antioxidants of the broilers have not been elucidated. Therefore, the current study was undertaken to investigate the antioxidant capacity of AA and/or ZB in tissues of broiler birds reared under hot climate.

MATERIALS AND METHODS

Birds, feed and additives used in the main experiment: A total of 200 day old broiler chicks (Ross 308; 40±3 g b.wt.) were purchased from Al Waziyah Poultry Company, Al-Nafjan Contracting Establishment, Eastern province, Dammam, Saudi Arabia. Birds were assigned to four treatments and housed in an open sided poultry house at the Research Station, King Faisal University, Al-Ahsa, Saudi Arabia (Ismail *et al*., 2013). The pens provided a floor area of 1.5 m^2. Each treatment constitutes 5 replicates (10 birds/replicate). The ambient temperature and relative humidity were monitored daily (39±2°C). 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 (1-42 day). AA (1 g/kg basal diet; T_2), ZB (100 mg/kg basal diet; T_3) and their combination (T_2 and T_1; T_4) were provided to heat stressed birds (Ismail *et al*., 2013).

Sampling: At the end of the experiment, liver, heart and kidney tissues of our previous experiment (Ismail *et al*., 2013) have been collected and stored frozen at -20°C until used in the current study for analysis of biochemical markers of oxidative stress. All experimental procedures and management conditions used in this study were approved by Committee of ethics of Scientific Research, King Faisal University, DSR130029.

Determination of hepatic antioxidant enzymes, thiobarbituric acid reactive substances (TBARS) and reduced glutathione: One gram of liver, heart and kidney tissues was homogenized in 5 mL of cold 20 mM HEPES buffer, pH 7.2, containing 1 mM EGTA, 210 mM mannitol and 70 mM sucrose. After centrifugation (1500×g per 5 min) at 4°C, the supernatants were removed and stored frozen at -80°C until the time of analysis of SOD. Another 1 g of the same tissues was homogenized in 5 mL of cold buffer of 50 mM potassium phosphate buffer, pH 7, containing 1 mM EDTA. After centrifugation (10.000×g per 15 min) at 4°C, the supernatants were removed and stored frozen at -80°C until the time of analysis of CAT, GPx, GST, GSH and GR.

The extent of lipid peroxidation in terms of MDA was measured by mixing 1 g of the examined tissues with RIPA buffer (Item No. 10010263, Cayman chemical company, USA). After homogenization, sonication and centrifugation (1600×g per 10 min), the supernatants were removed and stored frozen at -80°C until the time of analysis. The activities of CAT (nmol min^{-1} g^{-1} tissue; Cayman Chemical Company, USA, Catalog No. 707002), GPx (nmol min^{-1} g^{-1} tissue; Cayman Chemical Company, USA, Catalog No.703102), SOD (U g^{-1} tissue; Cayman Chemical Company, USA, Catalog No. 706002), GR (nmol min^{-1} g^{-1} tissue; Cayman, USA, Cat No. 703202) GST (nmol min^{-1} g^{-1} tissue; Cayman Chemical Company, USA, Catalog No. 703302) and concentrations of GSH (μM; Cayman Chemical Company, USA, Catalog No. 703002) and TBARS (μM; Cayman Chemical Company, USA, Catalog No. 10009055) were determined by ELISA reader (Absorbance Microplate Reader ELx 800TM BioTek®, USA). Results were calculated according to the manufacturer instructions.

Statistical analysis: All data was presented as Mean±Standard Deviation of using one way analysis of variance (ANOVA). All tests were performed using computer package of the statistical analysis system (SAS., 2002).

RESULTS

The data summarized in Table 1 indicated that, heat stress birds treated with AA, ZB and their combination showed significant decrease in MDA concentration of liver (19.0±3.4, 13.4±5.1, 18.0±1.1 μM), heart (7.5±2.1, 8.4±2.5, 5.7±4.1 μM) and kidney (14.2±2.9, 13.2±3.2, 10.6±3.1 μM), respectively compared to control untreated birds (53.2±4.1, 39.1±5.1, 40.5±3.5 μM) for 6 weeks. The concentrations of GSH in liver, heart and kidney tissues of heat stressed broiler chicken supplemented with AA and/or ZB for 6 weeks are presented in Table 1. The current findings indicated that, there were no significant changes in GSH concentrations of all experimental groups. However, hepatic CAT activity (Table 2) was only increased significantly in heat stressed birds treated with AA (60.5±2.5 nmol min^{-1} g^{-1} tissue) compared to control untreated heat stressed birds (49.0±3.1) and other heat stressed birds treated with either ZB (40.6±7.0) or combination of AA and ZB (47.9±7.6). The data presented in Table 2 indicated that, heat stress birds treated with AA, ZB and their combination showed significant increase in GPx activities of liver (757.4±11.5, 583.2±11.1, 579.5±13.6 nmol min^{-1} g^{-1} tissue), heart (300.5±5.4, 303.3±3.0, 306.6±5.0 nmol min^{-1} g^{-1} tissue) and kidney (1285.0±12.9, 1262.9±12.5,

Table 1: Tissue malondialdehyde and tissue reduced glutathione (μM) concentration of heat stressed broiler chicken supplemented with ascorbic acid and/or zinc bacitracin for 6 weeks

	Treatments			
Parameters	T_1	T_2	T_3	T_4
MDA (μM)				
Liver	53.2±4.1	19.0±3.4*	13.4±5.1*	18.0±1.1*
Heart	39.1±5.1	7.5±2.1*	8.4±2.5*	5.7±4.1*
Kidney	40.5±3.5	14.2±2.9*	13.2±3.2*	10.6±3.1*
GSH (μM)				
Liver	14.7±2.1	13.4±1.6	11.3±2.9	11.4±3.1
Heart	13.8±2.2	11.3±1.9	11.5±1.8	13.9±2.3
Kidney	10.1±1.4	11.4±1.7	10.4±1.6	10.2±1.8

T1: Control group untreated heat stressed birds, T2: Heat stressed birds treated with ascorbic acid, T3: Heat stressed birds treated with zinc bacitracin, T4: Heat stressed birds treated with combination of ascorbic acid and bacitracin, Values are Mean±SD, *Statistically significant when compared to control (group I) at $p<0.05$, MDA: Malondialdehyde and GSH: Reduced glutathione

Table 2: Tissue catalase, glutathione peroxidase, superoxide dimutase, glutathione-S transferase and glutathione reductase activity of heat stressed boiler chicken supplemented with ascorbic acid and/or zinc bacitracin for 6 weeks

	Treatments			
Parameters	T_1	T_2	T_3	T_4
CAT (nmol min^{-1} g^{-1} tissue)				
Liver	49.0±3.1	60.5±2.5*	40.6±7.0	47.9±7.6
Heart	41.7±4.1	36.5±6.1	29.7±8.2	49.0±4.4
Kidney	37.1±4.9	44.2±2.9	43.5±2.5	43.6±1.1
GPx (nmol min^{-1} g^{-1} tissue)				
Liver	448.7±10.3	757.4±11.5*	583.2±11.1*	579.5±13.6*
Heart	234.9±6.8	300.5±5.4*	303.3±3.0*	306.6±5.0*
Kidney	929.6±10.5	1285.0±12.9*	1262.9±12.5*	1259.5±10.1*
SOD (U g^{-1} tissue)				
Liver	0.5±0.1	0.4±0.1	0.3±0.1	0.5±0.1
Heart	0.4±0.1	0.3±0.1	0.4±0.1	0.3±0.1
Kidney	0.4±0.1	0.4±0.1	0.5±0.1	0.3±0.1
GST (nmol min^{-1} g^{-1} tissue)				
Liver	96.4±7.3	158.7±6.1*	85.1±7.1	98.6±5.5
Heart	23.9±5.1	51.1±2.1*	53.2±1.1*	43.4±3.2
Kidney	109.3±3.5	112.5±4.1	118.2±5.1	115.6±5.2
GR (nmol min^{-1} g^{-1} tissue)				
Liver	1537.0±10.1	2245.1±11.5*	1142.3±12.1	1120.0±13.4
Heart	441.0±11.9	820.1±10.2*	816.9±6.1*	800.3±11.3*
Kidney	1647.0±10.2	1659.0±13.2	1645.7±11.2	1651.2±11.2

T1: Control group untreated heat stressed birds, T2: Heat stressed birds treated with ascorbic acid, T3: Heat stressed birds treated with zinc bacitracin, T4: Heat stressed birds treated with combination of ascorbic acid and bacitracin, Values are Mean±SD, *Statistically significant when compared to control (group I) at $p<0.05$, CAT: Catalase, GPx: Glutathione peroxidase, SOD: Superoxide dismutase, GST: Glutathione-S transferase and GR: Glutathione reductase

1259.5±10.1 nmol min^{-1} g^{-1} tissue), respectively compared to control untreated birds (448.7±10.3, 234.9±6.8, 929.6±10.5 nmol min^{-1} g^{-1} tissue) for 6 weeks. The activities of SOD in liver, heart and kidney tissues of heat stressed broiler chicken supplemented with AA and/or ZB for 6 weeks are presented in Table 2. The current findings indicated that, there were no significant changes in SOD activities of all experimental groups. The activities of hepatic GST were significantly increased in heat stressed bird treated with AA only 158.7±6.1 nmol min^{-1} g^{-1} tissue compare to control untreated heat stressed birds (96.4±7.3 nmol min^{-1} g^{-1}) (Table 2). However heart GST activity was increased significantly in heat stress birds treated with AA (51.1±2.1 nmol min^{-1} g^{-1}) or ZB (53.2±1.1 nmol min^{-1} g^{-1}) compare to control (23.9±5.1 nmol min^{-1} g^{-1}), respectively (Table 2). The present findings indicated also that, the activities of hepatic GR were significantly increased in heat stressed bird treated with AA only (2245.1±11.5 nmol min^{-1} g^{-1} tissue) compare to control untreated heat stressed birds (1537.0±10.1 nmol min^{-1} g^{-1}). However heart GR activity was increased significantly in heat stress birds treated with AA (820.1±10.2 nmol min^{-1} g^{-1}) or ZB (816.9±6.1 nmol min^{-1} g^{-1}) or their combination (800.3±11.3 nmol min^{-1} g^{-1}) compare to control (441.0±11.9 nmol min^{-1} g^{-1}), respectively (Table 2).

DISCUSSION

Oxidative stress is generated when pro-oxidants overwhelms the antioxidants system of the body (Sies, 1991). Different stressors induced free radicals production and decreased the antioxidant system creating a case of oxidative stress and subsequent tissues damage. During

heat stress, the birds increased evaporative cooling, metabolism and energy consumption to maintains optimal body temperature (Gomez *et al*., 2002). Mobilization of lipids from the stored fats compensates the extra needs of energy. MDA level is positively correlated with lipid peroxidation (Whittow, 1994). The significant higher level of MDA in untreated heat stressed broilers indicated that higher level of free radicals were generated and overwhelms the antioxidant status of the birds. 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 Yardibi and Turkay (2008) and Puthpongsiriporn *et al*. (2001) in heat stressed laying hen. The lower levels of serum MDA in birds treated with AA and/or ZB either compared to heat stressed untreated birds, suggested that both additives acts as antioxidants and ameliorates the detrimental effect of heat stress. The significant reduction of MDA in ascorbic acid treated birds comes in accordance with Erdogan *et al*. (2005) and El-Shaieb *et al*. (2009) in broiler chicken. Superoxide radical is a highly reactive cytotoxic agent and is the primary reactive oxygen species. It is converted to hydrogen peroxide by SOD. Afterwards, hydrogen peroxide is converted to molecular oxygen and water by either CAT or GPx. Additionally, GPx can reduce lipid and hydroperoxides that are highly cytotoxic products. It is clear that, SOD, CAT and GPx are the principal components of the enzymatic antioxidant system able to neutralize the oxidative stress. GSH is the main non enzymatic antioxidant components which has the ability to conjugated with xenobiotic substances, a reaction catalyzed by GST. GSH can also directly scavenge reactive oxygen species and the resulting oxidized glutathione can be regenerate GSH through NADPH glutathione reductase (GR) system (El-Bahr, 2013). CAT and GPx are participated in the reaction of transformation of hydrogen peroxide to water thereby protects the cells from deleterious effect of hydrogen peroxide (Davies, 1995; Altan *et al*., 2003; Seven *et al*., 2009). The current findings indicated a significant increase of hepatic CAT activity only in heat stressed birds tratted with AA compared to other groups, however, AA, ZB and combination was able to increase the GPx activity of heat stressed birds in all examined tissues. This indicated that, AA was effective than ZB and combination as antioxidant because the reaction of transformation of hydrogen peroxide to water was catalyzed by two enzymes CAT and GPx and not GPx only as in case of ZB and combination. This suggestion is underlined also by the findings of the present study which indicated a significant increase of hepatic GST and GR activities only in heat stressed birds treated with AA compared to other groups while heart GST and GR activities were increased significantly in heat stressed birds either treated with AA or ZB. It was clear also that liver antioxidant enzymes were sensitive to AA than ZB and their combination which might suggests possible receptor specificity. Therefore, further studies are recommended to clarify this concept. Similar results were demonstrated that Vitamin C supplementation increased the antioxidant defense and ameliorate the oxidative stress of heat stressed broiler (Seven, 2008; Ismail *et al*., 2013) and white leghorn layers (Panda *et al*., 2008) and others (Schmeling and Nockels, 1978; Kutlu and Forbes, 1993; Sahin *et al*., 2002; Panda *et al*., 2008) demonstrated that, heat stress stimulates the biosynthesis and secretion of corticosteroids and this can be antagonized by dietary supplementation of ascorbic acid. The present study confirmed the previous one (Ismail *et al*., 2013) in that, AA and ZB were effective against heat induced oxidative stress in broiler chickens and AA was more efficient than ZB and combination for activation of enzymatic antioxidants in liver, heart and kidney tissues of broiler chickens.

CONCLUSION

The current study demonstrated that, ascorbic acid and/or zinc bacitracin improved the liver, heart and kidney antioxidant capacities of heat stressed birds with varying efficiency. Hepatic antioxidant activity was more sensitive to ascorbic acid than zinc bacitracin and their combination whereas responses of heart and kidney tissues to all additives was the same. Ascorbic acid is recommended to be supplemented in chicken diet as antioxidant agent against heat induced oxidative stress in broiler chickens. Further studies at receptors and molecular levels are recommended to clarify the actual mechanism of action of ascorbic acid.

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