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ORIGINAL PAPER

Cellular antioxidant enzyme activity and biomarkers for oxidative stress are affected by heat stress

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Abstract

Heat stress (HS) causes oxidative stress and cellular changes in an attempt to detoxify the harmful effects of reactive oxygen species (ROS). However, how ROS affect different organs in chickens under acute and chronic HS is relatively unknown. We investigated the cellular enzyme activity and biomarker changes in the liver and *Pectoralis* (*P*) *major* muscle in broiler chickens subjected to both acute and chronic HS. Forty-eight broiler chickens at 14 days old were randomly assigned to either 25 °C (control) or 35 °C (heat-stressed) for 12 days. Five birds per treatment at 1 and 12 days post-HS were euthanized, and the liver and *P. major* muscle were sampled. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH), glutathione reductase (GR), glutathione S-transferase (GST) activity as well as 8-hydroxy-2′-deoxyguanosine (8-OHdG), advanced glycation end product (AGE), malondialdehyde (MDA), and protein carbonyl (PCO) were analyzed as biomarkers for DNA, carbohydrate, lipid, and protein oxidation, respectively. The SOD, CAT, and GSH-GPx activity levels in the liver and the *P. major* muscle changed under HS; however, some of the changes were tissue-specific or dependent on the duration of the HS. There were increased liver 8-OHdG during chronic HS and also increased liver AGE levels during both acute and chronic HS indicating significant carbohydrate and DNA oxidations. In the *P. major muscle*, we observed significant increases in lipid peroxidation and protein oxidation which may reflect that this tissue is less resilient to oxidative damage under heat stress. We show that heat stress caused tissue-specific changes to levels of oxidation biomarkers in chicken.

Keywords Heat stress · Oxidative stress · DNA oxidation · Lipid peroxidation · Protein oxidation

Introduction

Heat stress (HS) in growing birds affects performance (Temim et al. 1999), amino acid digestibility (Habashy et al. 2017a), dietary protein retention (Geraert et al. 1996; Habashy et al. 2017b), and mRNA expression of genes that encode for oxidants and antioxidants (Mujahid et al. 2007; Habashy et al. 2018) causing an imbalance between oxidant and antioxidant defense systems. HS promotes the generation of reactive oxygen species (ROS) which causes oxidative stress (Lu et al. 2010).

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ROS in general causes oxidative damage to DNA, proteins, and lipids (Dröge 2002). ROS represent partially reduced oxygen metabolites such as superoxide anions, hydrogen peroxide, and hydroxyl radicals (Thannickal and Fanburg 2000). There are a number of both enzymatic and non-enzymatic systems that are involved in the elimination of ROS (Lu et al. 2010). One of the non-enzymatic antioxidants is glutathione (GSH) and the enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Cadenas and Davies 2000). GSH serves as an electron donor for GPx which reduces hydrogen peroxide to water (Hayes and McLellan 1999). By donating an electron, GSH is oxidized to glutathione disulfide (GSSG). Glutathione disulfide can be reduced back to GSH by glutathione reductase (GR) (Srinivasan et al. 1997). Therefore, the cellular GSH to GSSG ratio plays an essential role in the detoxification of ROS and maintaining redox homeostasis. Glutathione S-transferase (GST) plays an important role in protecting cells and tissues from oxidative damage (Hayes et al. 2005) by accelerating the reaction between GSH and different electrophilic xenobiotics (Zhu et al. 2013).

Biomarkers have been used as proxies to determine the extent of oxidation stress in tissues. Protein carbonyl (PCO), malondialdehyde (MDA), 8-hydroxy-2′-deoxyguanosine (8-OHdG), and advanced glycation end product (AGE) have been used as indicators of protein, lipid, DNA, and carbohydrate oxidation, respectively (Levine et al. 1994; Mujahid et al. 2009; Ismail et al. 2013; Sultana et al. 2013). Since oxidative stress is caused by several factors, the relative importance of various responses to the type of causal factor will be important in designing mitigation strategies to reduce its effect. Understanding the relative sensitivity of tissues to oxidative stress may assist in the design of mitigation strategies to reduce this effect on that particular tissue. There are several reports on short-term response to HS, but limited data on long-term effects of HS (Geraert et al. 1996; Azad et al. 2010; Sun et al. 2015; Habashy et al. 2017a, b). Our objective was to study the cellular enzyme activity and biomarker changes in the liver and *Pectoralis major* muscle in meat-type chickens exposed to both short- and long-term heat stress.

Material and methods

Experimental design and animals

Research on live chickens met the guidelines approved by the institutional animal care and use committee of the University of Georgia. A total of 48 male broilers (Cobb500) were divided into two treatments and raised under either constant normal (25 °C; 40–50% humidity) or high temperature (35 °C; 40–50% humidity) from day 14–26 of age in individual cages ($L = 30.48$ cm × $B = 60.96$ × $H = 45.72$). The birds were fed ad libitum on a diet containing 18.7% crude protein (CP) and 3560 kcal ME/kg. At day 15 (after 1 day of HS exposure) and day 26 (after 12 days of HS exposure) of age, birds were humanely euthanized by cervical dislocation and the *P. major* muscle and liver tissue were collected from five birds from each treatment and were immediately placed in liquid nitrogen and later stored at − 86 °C.

Determination of superoxide dismutase (U/mL)

Approximately 0.2 g of the *P. major* muscle and liver tissue were homogenized in 1 mL of cold 20 mM HEPES buffer, pH 7.2, containing 1 mM EGTA, 210 mM mannitol, and 70 mM sucrose. After centrifugation at 1500×*g* for 5 min at 4 °C, the supernatants were used for determining superoxide dismutase (SOD) activity level (Cayman Chemical Company, Ann Arbor, MI, USA, item no.706002). Activity was measured using Spectra Max 5 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 460 nm.

Determination of catalase (nmol/min g tissue), glutathione S-transferase (nmol/min/mL), and glutathione reductase (nmol/min/mL)

About 0.2 g of the *P. major* muscle and liver tissue were homogenized in 1 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 − 86 °C until the time of analysis of CAT, GST, and GR. The activity of CAT (Cayman Chemical Company, Ann Arbor, MI, USA, item no. 707002), GST (Cayman Chemical Company, Ann Arbor, MI, USA, item no. 703302), and GR (Cayman Chemical Company, Ann Arbor, MI, USA, item no. 703202) were measured with a Spectra Max 5 microplate reader (Molecular Devices, Sunnyvale, CA) at 540, 340, and 340 nm, respectively.

Determination of glutathione peroxidase (nmol/min/mL)

Approximately 0.2 g of the *P. major* muscle and liver tissue were homogenized in 1 mL of cold 50 mM Tris-HCL, pH 7.5, 5 mM EDTA, and 1 mM DTT, and the supernatant was centrifuged at 10000×*g* for 15 min at 4 °C and used for determination of GPX activity (nmol/min/mL) (Cayman Chemical Company, Ann Arbor, MI, USA, item no. 703102). Activity was determined by the Spectra Max 5 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 340 nm.

Determination of glutathione (μM) and glutathione disulfide (μM)

About 0.2 g of the *P. major* muscle and liver tissue were homogenized in 1 mL of cold 50 mM phosphate buffer, pH 7, containing 1 mM EDTA. After centrifugation (10,000×*g* for 15 min) at 4 °C, the supernatants were removed and stored frozen at − 86 °C until the time of analysis of GSH and GSSG. The activities of GSH and GSSG (Cayman Chemical Company, Ann Arbor, MI, USA, item no. 703002) were determined by the Spectra Max 5 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 405 nm.

Determination of DNA oxidative damage (pg/mL)

DNA was extracted from the *P. major* muscle and liver tissue using Trizol reagent (Invitrogen Corp., Carlsbad, CA, USA) according to manufacturer's instructions and stored at − 20 °C until used. After that, DNA samples were converted to single-stranded DNA by incubation at 95 °C for 5 min and rapidly chilled at 4 °C. Then, DNA samples were digested using five units nuclease P1 (Sigma-Aldrich, Saint Louis, MO, USA, item no. 8630). One molar of Tris was used to adjust the pH to 7.5–8.5, and 5 μL alkaline phosphatase was added and the

product was incubated for 45 min at 37 °C. The lysate was then boiled for 10 min and stored in 4 °C until used. The DNA/RNA oxidative damage (Cayman Chemical Company, USA, item no. 589320) was determined by the Spectra Max 5 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 405 nm.

Determination of advanced glycation end product (μg/mL)

Approximately 0.1 g of the *P. major* muscle and liver tissue were homogenized in 1 mL of phosphate-buffered saline (PBS), pH 7.4, containing 10 μL of a protease inhibitor. After centrifugation at 12,000×*g* for 10 min at 4 °C, the supernatants were removed and used for measuring AGE activity (Cell Biolabs, San Diego, CA, USA, item no. STA-817). AGE activity was measured by the Spectra Max 5 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 450 nm.

Determination of malondialdehyde (μM)

About 25 mg of the *P. major* muscle and liver tissue were homogenized in 250 μL of RIPA buffer containing 1 μL

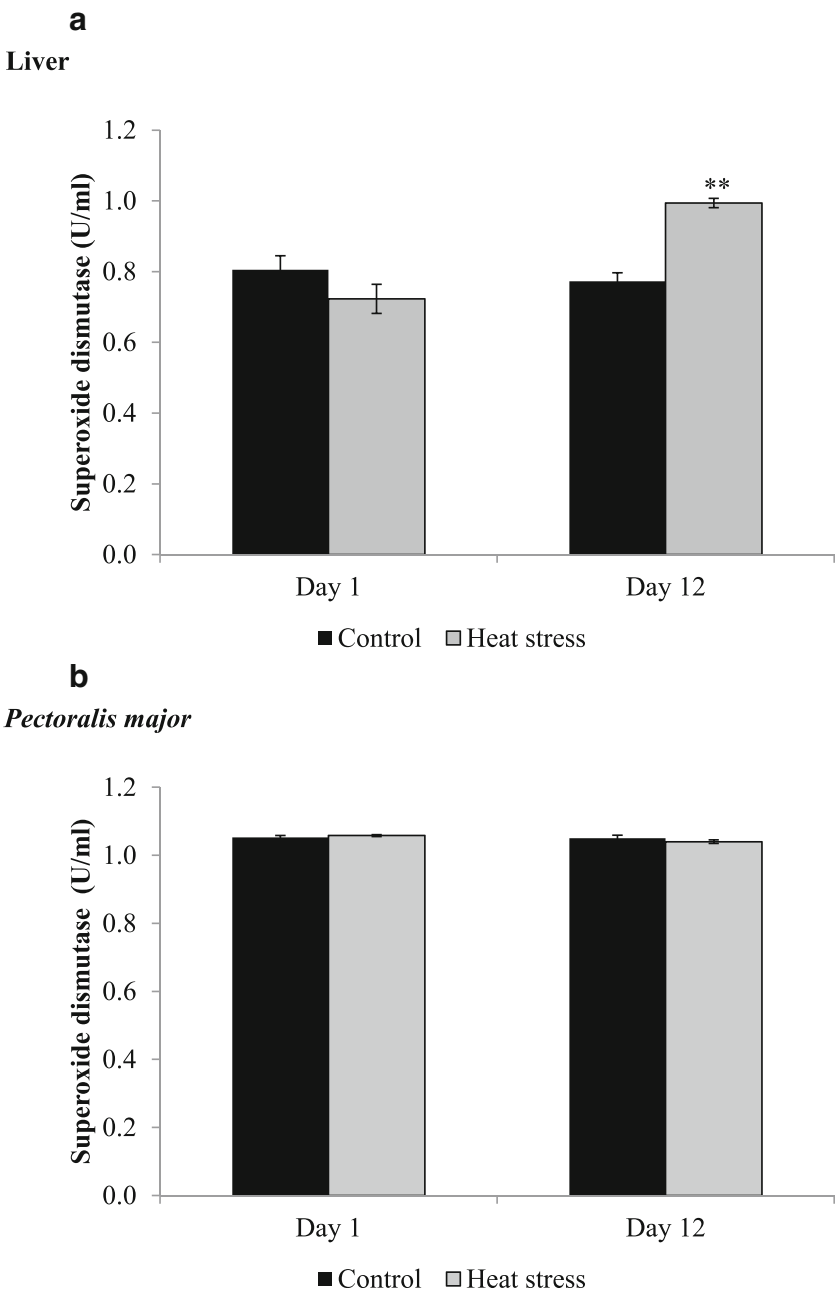

Fig. 1 Effect of heat stress on superoxide dismutase activity in the liver (**a**) and *Pectoralis major* (**b**) tissue of broiler (**$P < 0.01$)

protease inhibitors) and then centrifuged at 1,600×*g* for 10 min at 4 °C. The supernatants were then used for measuring the malondialdehyde (MDA) activity (Cayman Chemical Company, Ann Arbor, MI, USA, item no. 10009055). MDA activity was measured by the Spectra Max 5 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 540 nm.

Determination of protein carbonyl (nmol/mL)

Approximately 0.2 g of the *P. major* muscle and liver tissue were homogenized in 1 mL of cold 50 mM potassium phosphate buffer, pH 7, containing 1 mM EDTA. After centrifugation (10,000×*g* for 15 min) at 4 °C, the supernatants were removed and incubated with 10%

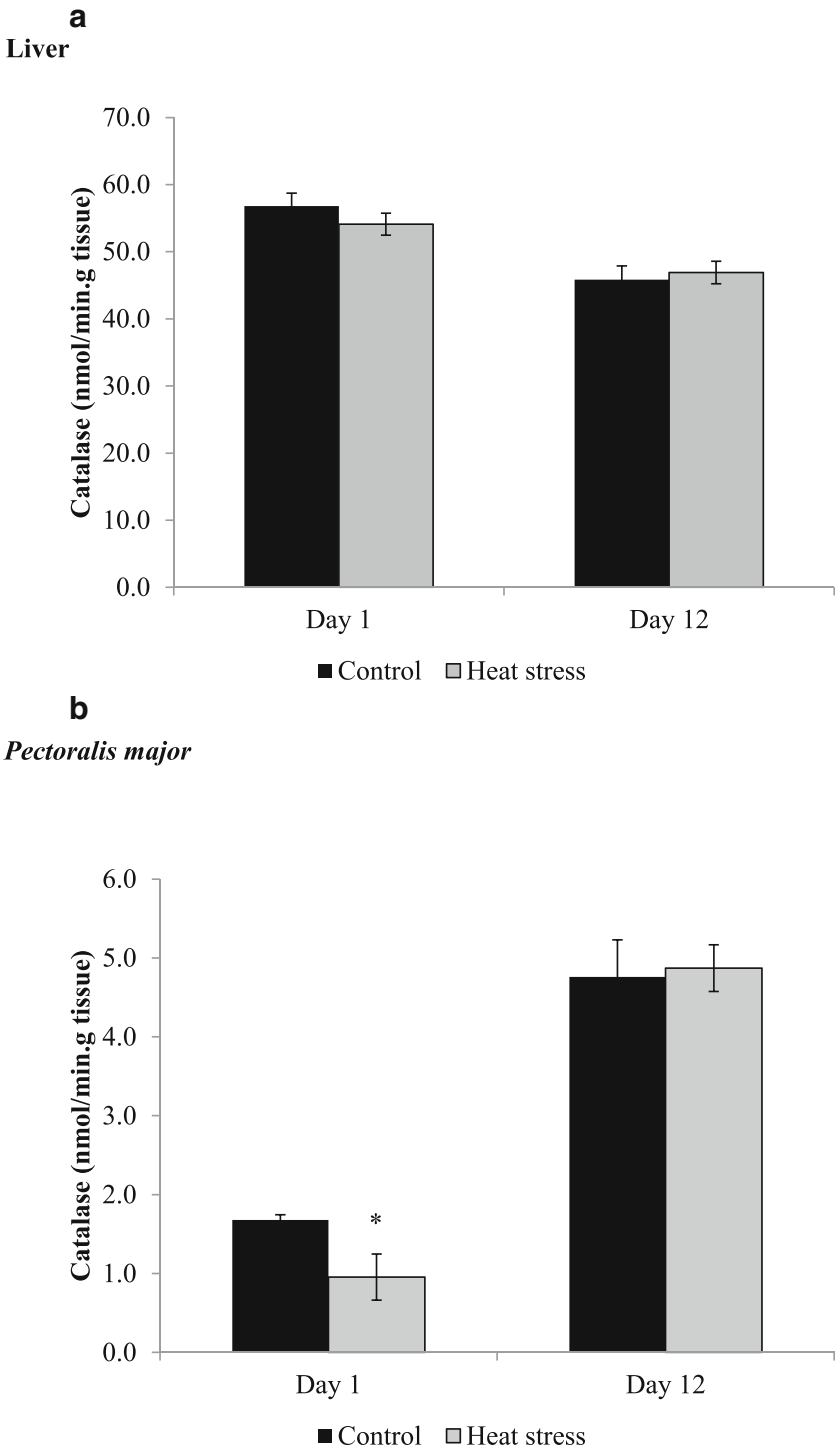

Fig. 2 Effect of heat stress on catalase activity in the liver (**a**) and *Pectoralis major* (**b**) tissue of broiler (*$P < 0.05$)

streptomycin sulfate stock solution for 15 min at room temperature and then centrifuged at 6,000×*g* for 10 min at 4 °C. Following centrifugation, the supernatants were used for determining the protein carbonyl content (Cayman Chemical Company, Ann Arbor, MI, USA, item no. 10005020). Activity was determined by the Spectra Max 5 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 360 nm.

Statistical analysis

Statistical analysis was performed separately for acute and chronic HS and each tissue. 8-OHdG, AGE, MDA, PCO, SOD, CAT, GPX, GSH, GR, GST, GSSG, and GSH/GSSG as dependent variable between treatment (HS and control groups) were analyzed using SAS 9.4 (2011), and the differences among treatments were tested using the Tukey test.

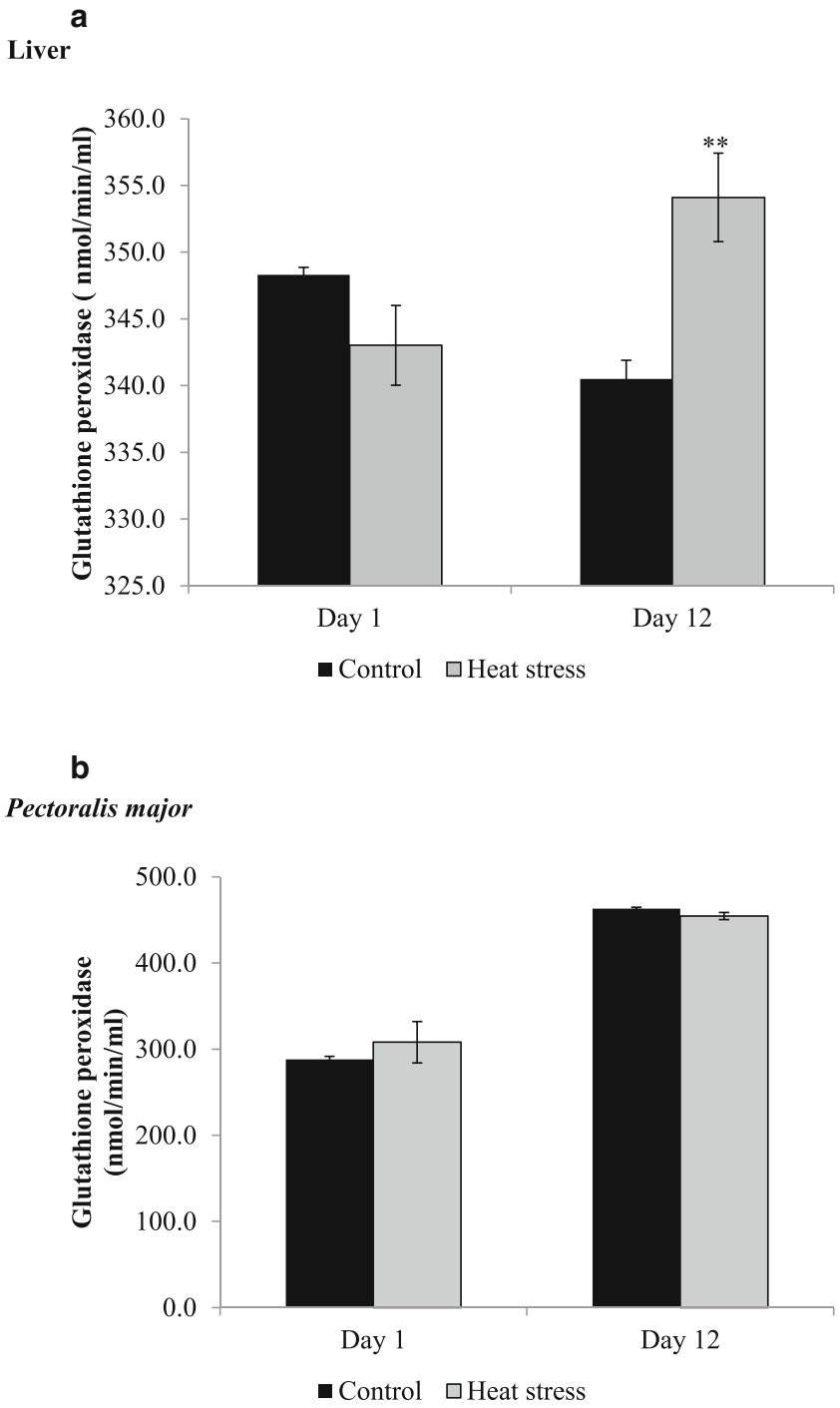

Fig. 3 Effect of heat stress on glutathione peroxidase activity in the liver (**a**) and *Pectoralis major* (B) tissue of broiler (**$P < 0.01$)

Results

Cellular antioxidant enzyme activity

The cellular levels of SOD, CAT, GPx, GSH, GR, GST, GSSG, and GSH/GSSG ratio are presented in Figs. 1, 2, 3, 4, 5, 6, 7, and 8. The SOD levels increased ($P < 0.0001$) in the liver only at 12 days post-HS. There were no changes ($P > 0.05$) in the SOD levels in the *P. major* muscle both at 1 and 12 days post-HS (Fig. 1). Also, heat-stressed birds showed a decrease ($P < 0.05$) in the CAT level at 1 day post-HS in the *P. major* muscle, but there were no changes ($P > 0.05$) in the CAT levels in the liver when birds were subjected to HS (Fig. 2). The liver levels of GPx did not change when birds were subjected to 1 day of HS; however, at 12 days post-HS, there was an increase ($P < 0.0029$) in the liver GPx level. In the *P. major* muscle, the level of GPx was similar between the heat-stressed birds and the controls both at 1 and 12 days post-HS (Fig. 3). The GSH levels in both the liver and *P. major* muscle were similar between the heat-stressed birds and control birds at 1-day treatment, but at 12 days post-treatment, the GSH levels decreased ($P < 0.001$) in the liver, but increased ($P < 0.0023$) in the *P. major* muscle of birds subjected to HS (Fig. 4). Tissue GR levels are shown in Fig. 5. Among heat-stressed birds, the GR activity increased ($P < 0.05$) at 1 day post-HS; however, by 12 days post-HS, the GR levels in the liver had decreased ($P < 0.01$) compared with the control birds. In the *P. major* muscle, GR increased ($P < 0.05$) only at 1 day post-HS. The GST level did not change in the liver and *P. major* at

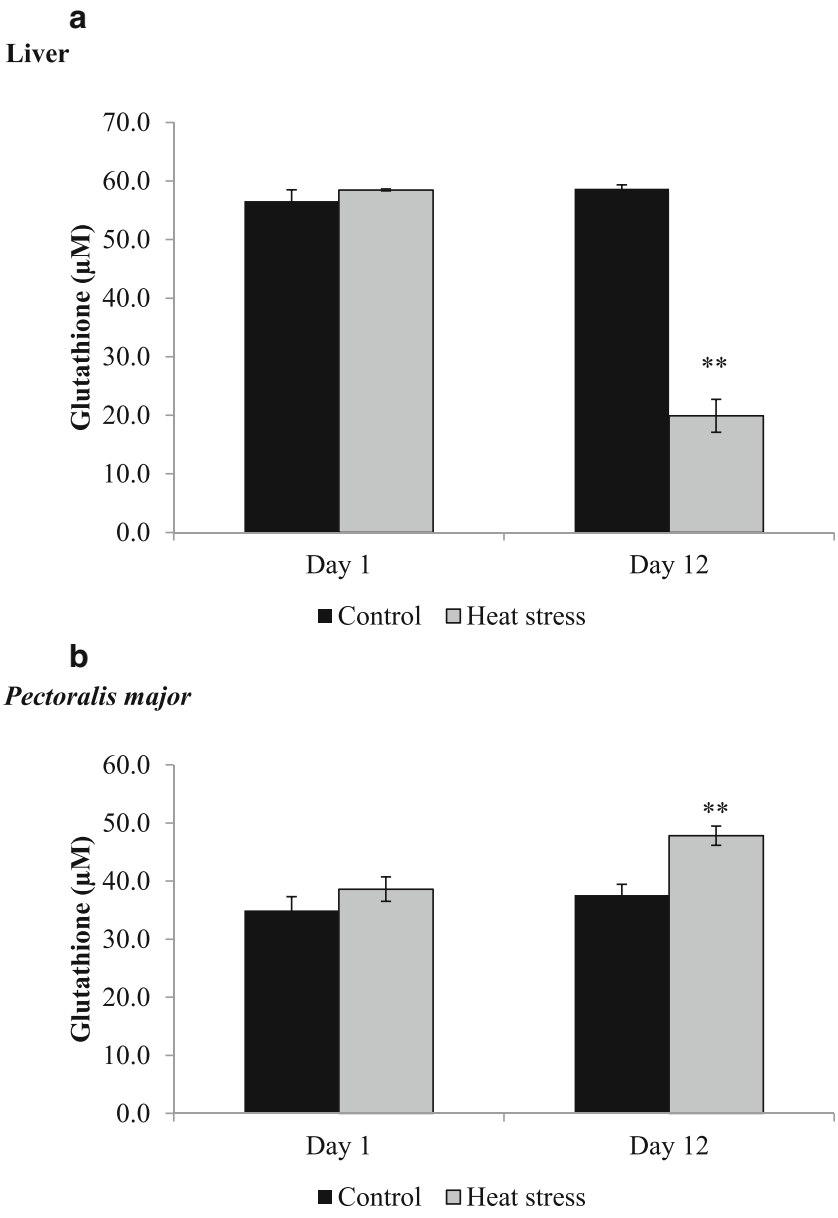

Fig. 4 Effect of heat stress on glutathione activity in the liver (**a**) and *Pectoralis major* (**b**) tissue of broiler (**$P < 0.01$)

1 d post-HS, but decreased ($P < 0.0001$) only in the liver at 12 days post-HS (Fig. 6). At 1 day post-treatment, GSSG levels increased ($P < 0.01$) in both the liver and *P. major* muscle in heat-stressed birds compared with the controls. The opposite effect was observed at 12 days post-treatment where the GSSG levels decreased ($P < 0.01$) in the heat-stressed birds compared with the control birds (Fig. 7). The GSH/GSSG ratio did not change in the liver under both acute and chronic heat stress; however, in the *P. major* muscle, the ratio decreased in the heat-stressed birds at 1 day post-HS, but increased at 12 days post-HS (Fig. 8).

Biomarkers

The biomarkers for DNA, carbohydrate, lipid, and protein oxidations in the liver and *P. major* muscle are presented in Figs. 9, 10, 11, and 12. Figure 9 shows that 8-OHdG was significantly increased ($P < 0.0007$) in the liver at 12 days

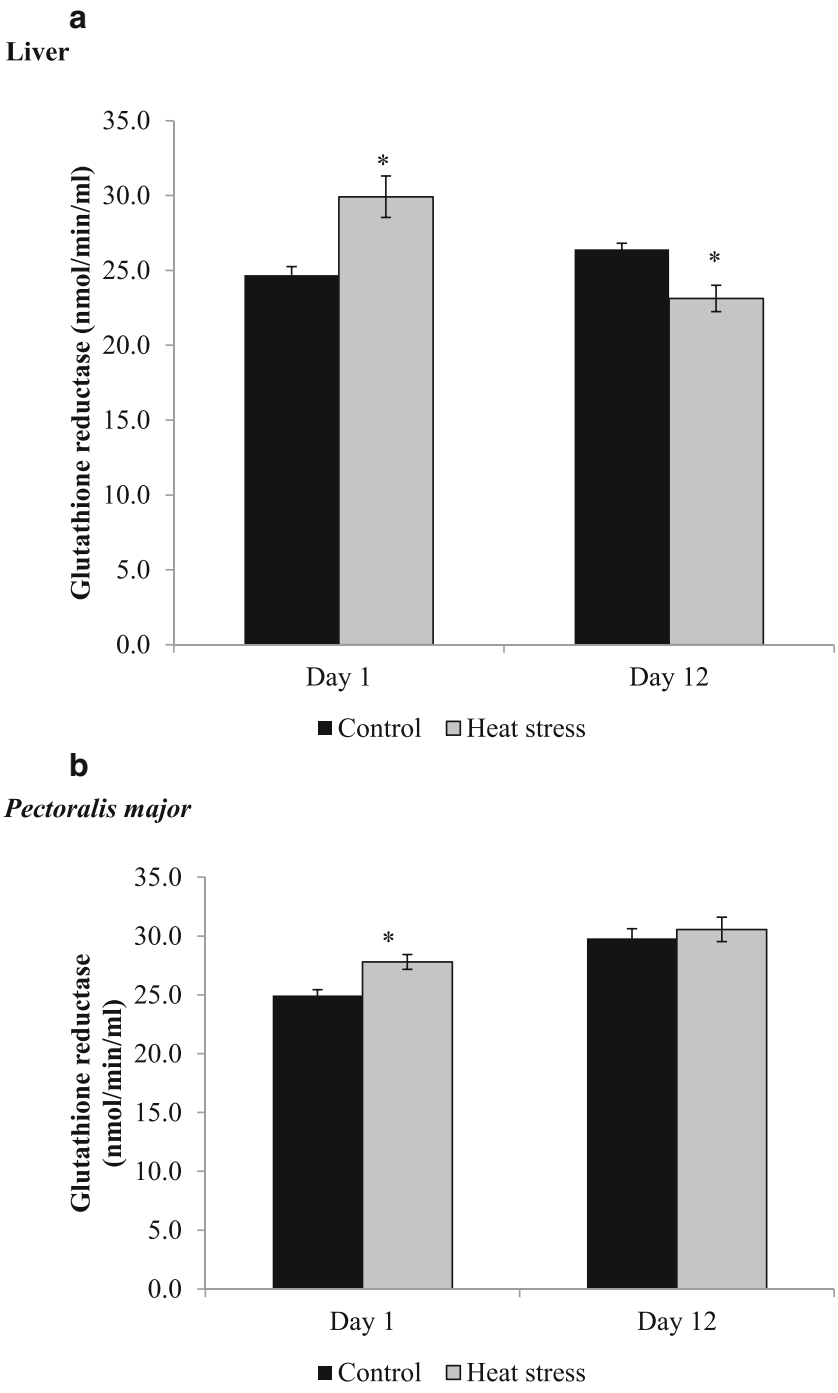

Fig. 5 Effect of heat stress on glutathione reductase activity in the liver (**a**) and *Pectoralis major* (**b**) tissue of broiler (*$P < 0.05$)

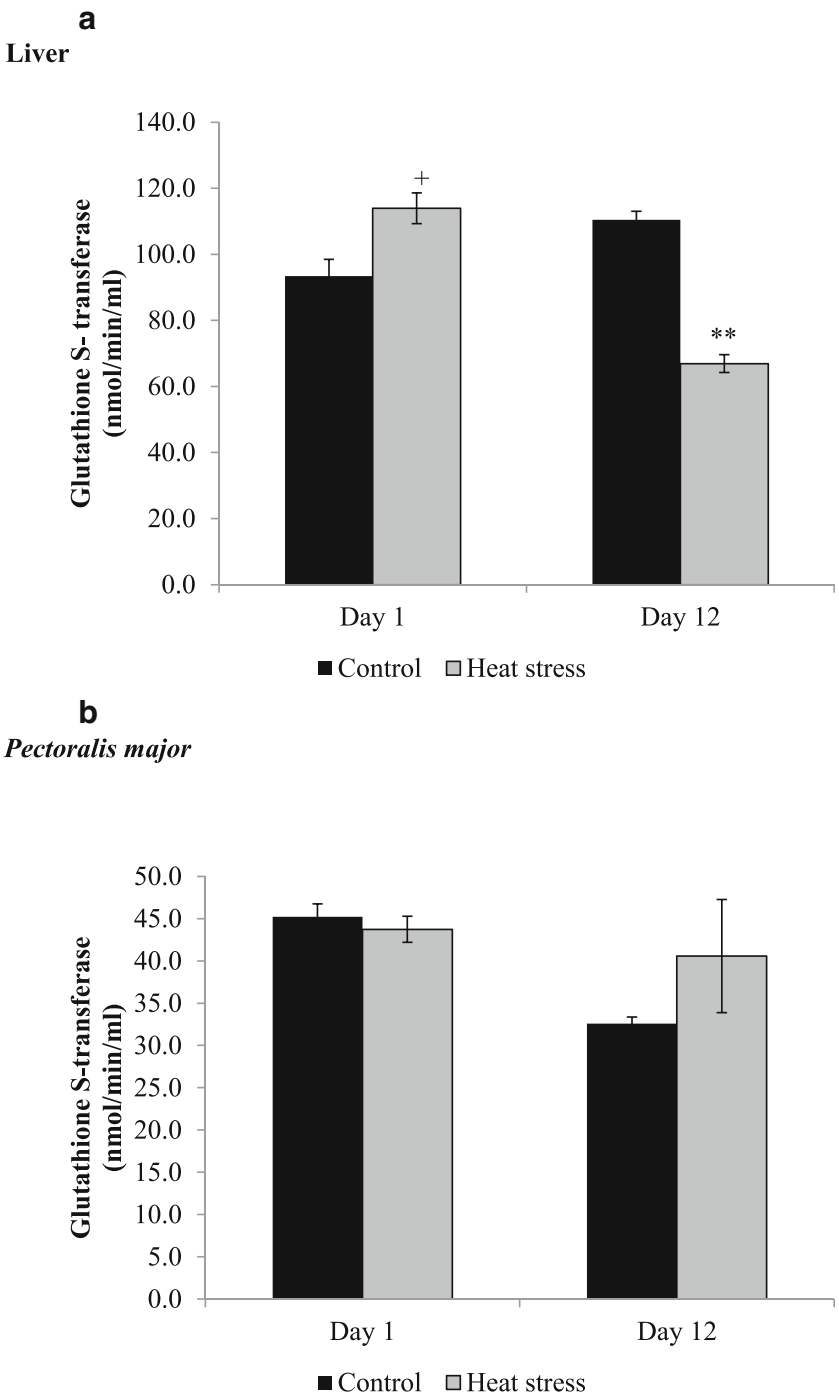

Fig. 6 Effect of heat stress on glutathione S-transferase activity in the liver (**a**) and *Pectoralis major* (**b**) tissue of broiler (**$P < 0.01$ and $^{+}P < 0.1$)

post-HS compared with their control counterparts, but in the *P. major* muscle, 8-OHdG did not change between the control and heat-stressed chickens. Chickens exposed to HS had significant increase in the activities of AGE in the liver at 1 ($P < 0.005$) and 12 days ($P < 0.008$) post-HS; however, in the *P. major* muscle, AGE did not change between the groups at 12 days post-HS (Fig. 10). The MDA activities in the liver did not differ between the treatment and control groups (Fig. 11) at both 1 and 12 days post HS. Meanwhile, in the *P. major* muscles, the levels of MDA were significantly increased after 1 ($P < 0.0075$) and 12 days ($P < 0.05$) post-HS (Fig. 11). The PCO levels in the liver increased significantly at 1 day post-HS, but not at 12 days post-HS, but in the *P. major* muscle, PCO levels significantly increased both at 1 ($P < 0.0040$) and 12 days ($P < 0.0002$) post-HS (Fig. 12).

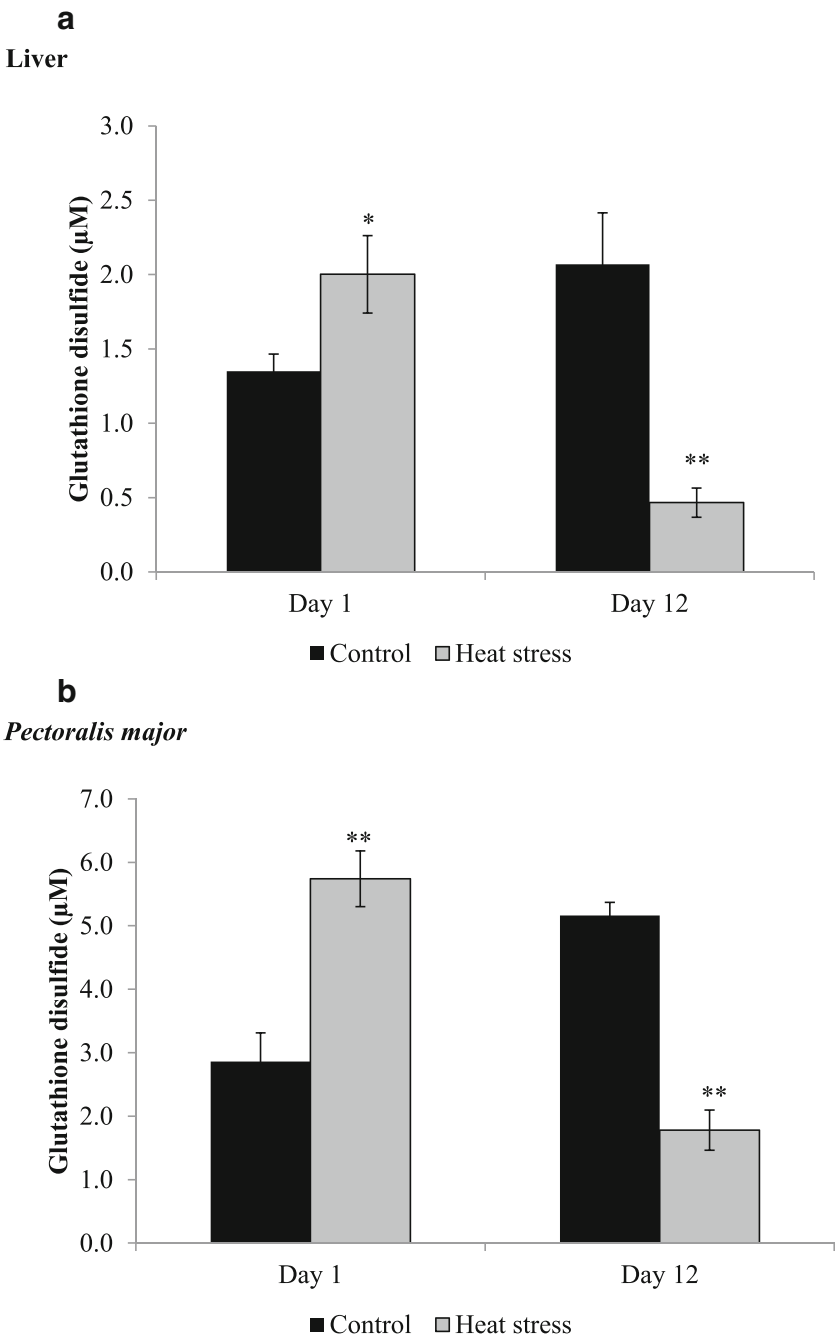

Fig. 7 Effect of heat stress on glutathione disulfide activity in the liver (**a**) and *Pectoralis major* (**b**) tissue of broiler (**$P < 0.01$ and *$P < 0.05$)

Discussion

Heat is one of the major stressors affecting the poultry industry, and several studies have indicated that HS has a negative impact on productivity (Habashy et al. 2017a), immune competence (Mashaly et al. 2004), and reproductive performance (Rozenboim et al. 2007). Based on physiological responses to heat stress, several mitigation strategies have been tried with varying success. An understanding of the cellular responses to HS could offer additional strategies to curtail the effects of HS in growing birds. There are several reports that demonstrate that HS causes oxidative stress. Habashy et al. (2018) have shown that mRNA expression of genes that encode for oxidants is altered by heat stress in a way that it putatively increases the cellular ROS activity.

Cellular antioxidant enzyme activity

The main cellular antioxidant enzymatic defense systems against ROS are SOD, GPx, and GR. They scavenge ROS and convert them to less reactive species. Heat stress has been shown to elevate the cellular level of ROS in several animal models (Kikusato and Toyomizy 2013). Reactive species comprise of superoxide,

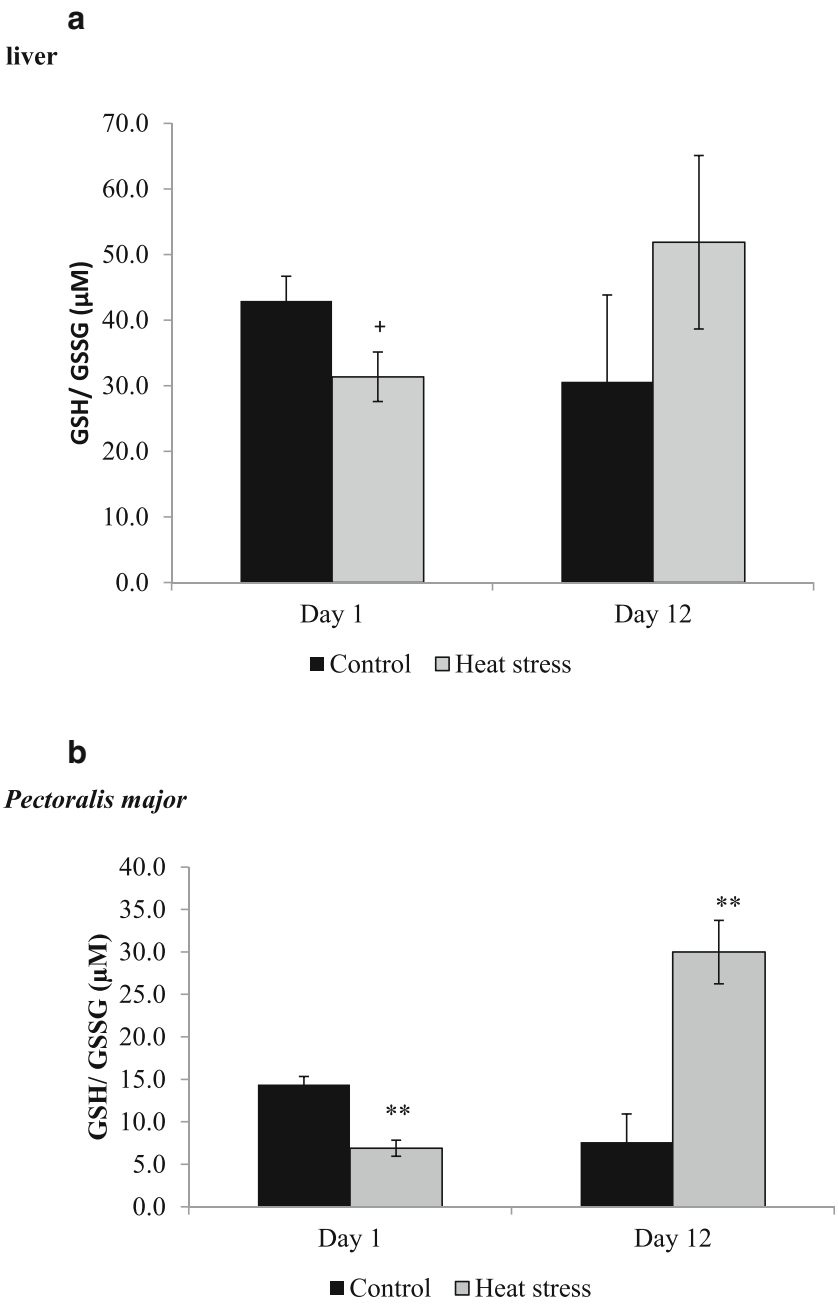

Fig. 8 Effect of heat stress on glutathione-glutathione disulfide ratio in the liver (**a**) and *Pectoralis major* (**b**) tissue of broiler (**$P < 0.01$ and $^{+}P < 0.1$)

hydroxyl, hydrogen peroxide, nitric oxide, and nitrogen dioxide (Halliwell 2001). SOD catalyzes the conversion of the superoxide radical to hydrogen peroxide (H_2O_2) (Fridovich 1997). The liver SOD levels increased under chronic heat stress but not under acute stress. Other researchers have also demonstrated the changes in liver SOD levels under HS (Lin et al. 2000; Yang et al. 2010; Tan et al. 2010). Huang et al. (2015) also showed that chronic heat stress led to increased plasma SOD levels in chickens. The SOD response to HS may depend on the duration of the stress and also the tissue, as SOD levels in the *P. major* muscle did not change under both acute and chronic HS. The tissue SOD level under heat stress may reflect the tissue's role in detoxification and tolerance for cellular oxidation. Increase in liver SOD observed in the current study may be due to the need to detoxify increased free radicals resulting from the heat stress. Both protein and lipid oxidation levels were also significantly elevated in the *P. major* muscle compared with the liver under chronic HS. Based on these observations, it seems that the liver tissue plays a larger role in scavenging free radicals. Hydrogen peroxide is an ROS that needs to be converted to water. Catalase enzymatically converts H_2O_2 to water and O_2 to protect the cells from the destructive effect of H_2O_2 (Halliwell 2001; Finkel and Holbrook 2000; Altan et al. 2003; Seven et al. 2009). In the current study, liver CAT levels did not change when birds were subjected to HS. However, when

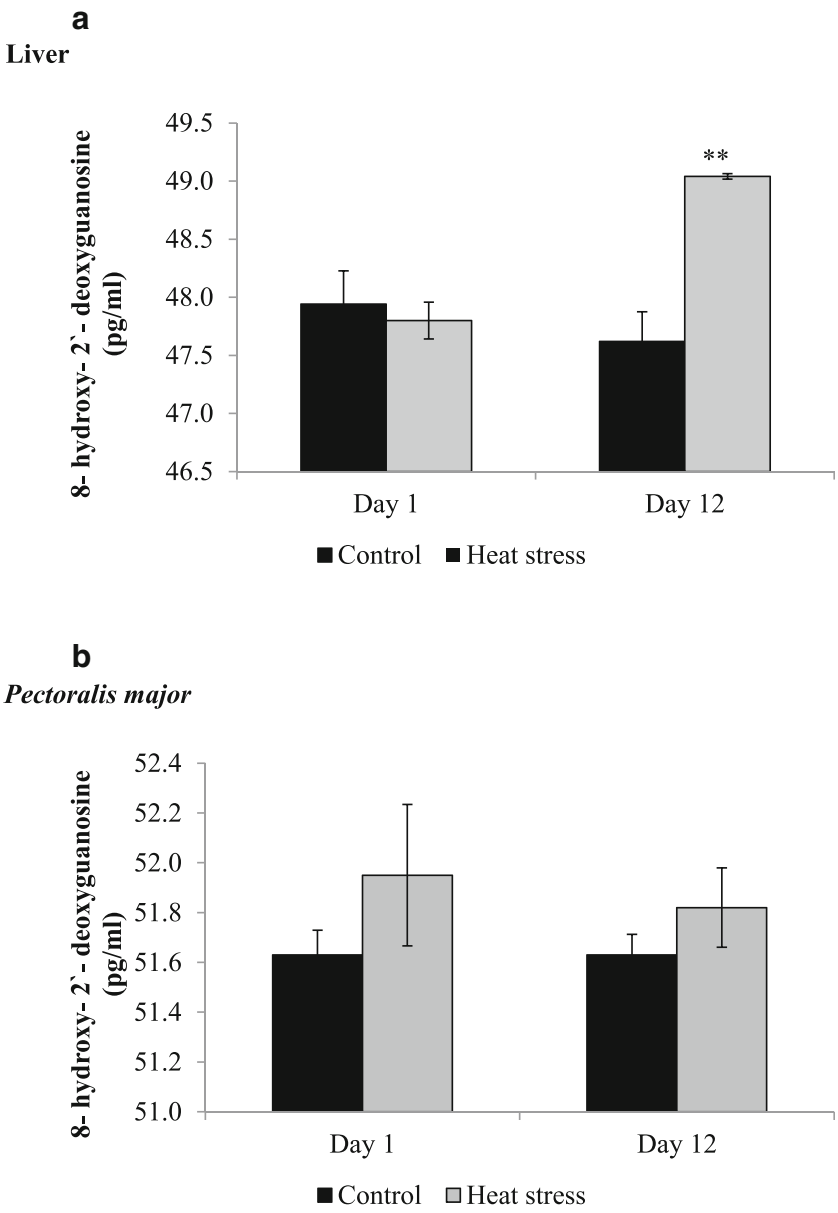

Fig. 9 Effect of heat stress on 8-hydroxy-2′-deoxyguanosine activity in the liver (**a**) and *Pectoralis major* (**b**) tissue of broiler (**$P < 0.01$)

Japanese quails were subjected to cyclical heat stress, liver CAT and GPx levels were reduced (Sahin et al. 2010). Baud et al. (2004) reported that increased H_2O_2 levels corresponded with decreased CAT activity in rat oligodendrocytes.

One of the principal redox pathways is the GSH-GPx enzyme system. Glutathione peroxidase and GSH coordinate to eliminate H_2O_2 and peroxynitrite as well as acting as a buffer to protect certain proteins against pathological modifications (Chen et al. 2015). GPX enzymes scavenge H_2O_2 by utilizing two hydrogens from two GSH molecules, thereby oxidizing GSH to GSSG. GSSG is reduced to GSH by using electrons donated by NADPH and catalyzed by GR (El-Bahr 2013). Liver GPx levels increased at 12 days post-HS, but there was no change observed in the *P. major* muscle. The GSH levels did not change in both tissues 1 day post-HS; however, at 12 days post-HS, the levels depended on the tissue. It appears that the activity levels of CAT, GPx, GR, and GSH are coordinated differently among tissues, possibly due to the tissue's relative tolerance to the damage caused by oxidative stress. Murphy and Kehrer (1989) reported that higher activities of GSH in the muscle of dystrophic chickens may reflect a higher degree of continuing oxidative stress in this tissue. In the current study, we have shown that there is a negative relationship between GSH and 8-OHdG, AGE in the liver tissue at day 12 post-HS, whereas the relationship between GSH and AGE, MDA, and PCO in the *P. major* muscle tissue at day 12 post-HS was positive. GSH can also bind to byproducts of lipid oxidation in a reaction catalyzed by GST (Cheng et al. 2001). From the current study, GST levels increased slightly in the liver at 1 day post-HS, but decreased

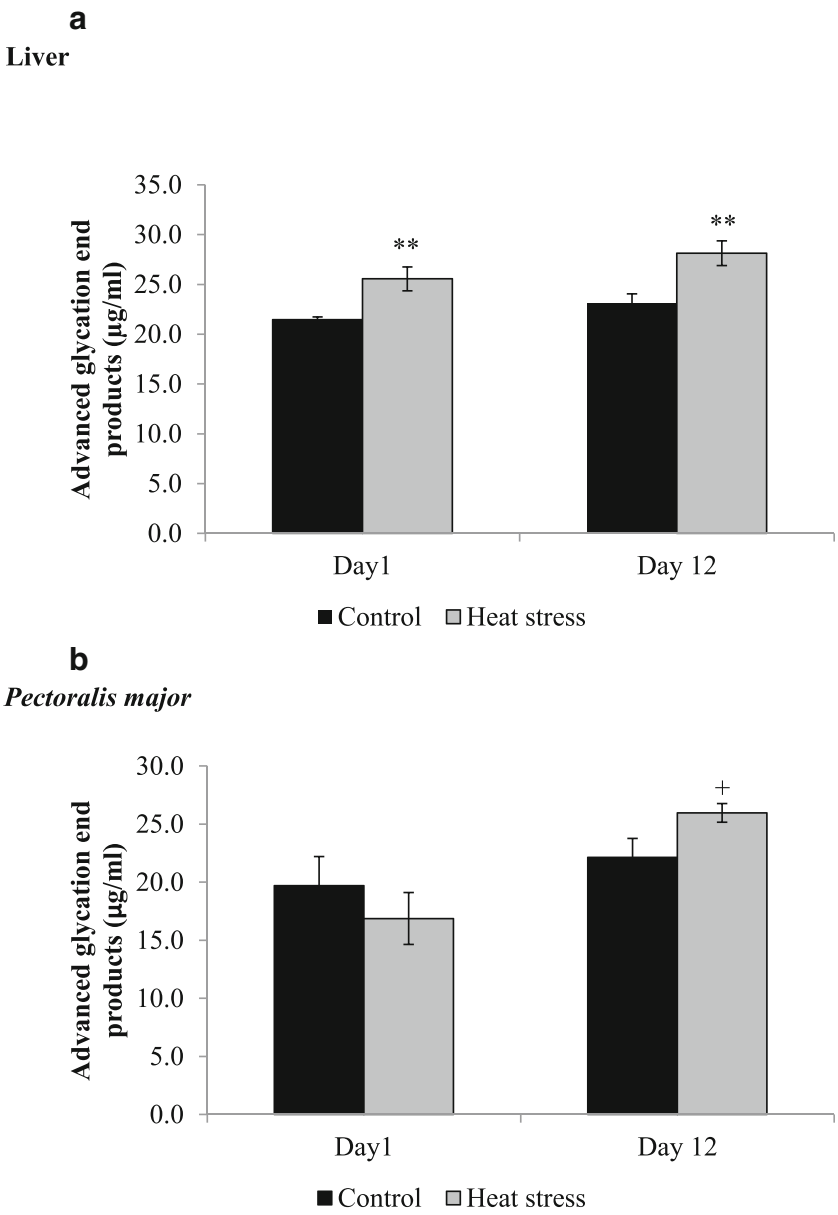

Fig. 10 Effect of heat stress on advanced glycation end product activity in the liver (**a**) and *Pectoralis major* (**b**) tissue of broiler (**$P < 0.01$ and $^{+}P < 0.1$)

when the HS continued up to 12 days. It is possible that the reduced GST at 12 days post-HS in the liver may be due to concurrent depletion in GSH. The GSH:GSSG ratio, often referred to as redox potential, is used as a measure of oxidative stress (Zitka et al. 2012). The level of GSSG increased in both the liver and *P. major* muscle with acute HS; however, under chronic stress, GSSG levels decreased probably due to increased detoxification of ROS. The redox potential was reduced under acute HS in both tissues because of the significant increase in GSSG. Under chronic HS, we observed an increase in the redox potential in the *P. major* muscle which may imply a relatively different enzymatic steady state aiming at detoxifying the muscle from ROS. Flohé (2013) cautioned that a GSH/GSSG equilibrium does not exist, but the ratio is a reflection of a steady state resulting from enzymatic use and regeneration of GSH which could be useful as a tool to unearth shifts in redox metabolism.

Biomarkers

Oxidative stress changes the balance between oxidants and antioxidants either by accumulation of ROS and/or depletion of antioxidants. Increased levels of ROS can alter DNA and RNA structures and gene expression (Wautier et al. 2001; Habashy et al. 2018). Several types of base damage by ROS have been demonstrated, but guanine is the most reactive of the nucleic acid bases (Barciszewski et al. 1999). Thus, the oxidized base 8-OHdG is the most abundant among the oxidized bases and therefore used as a biomarker for DNA oxidation (Helbock et al. 1999). In the current study, liver 8-

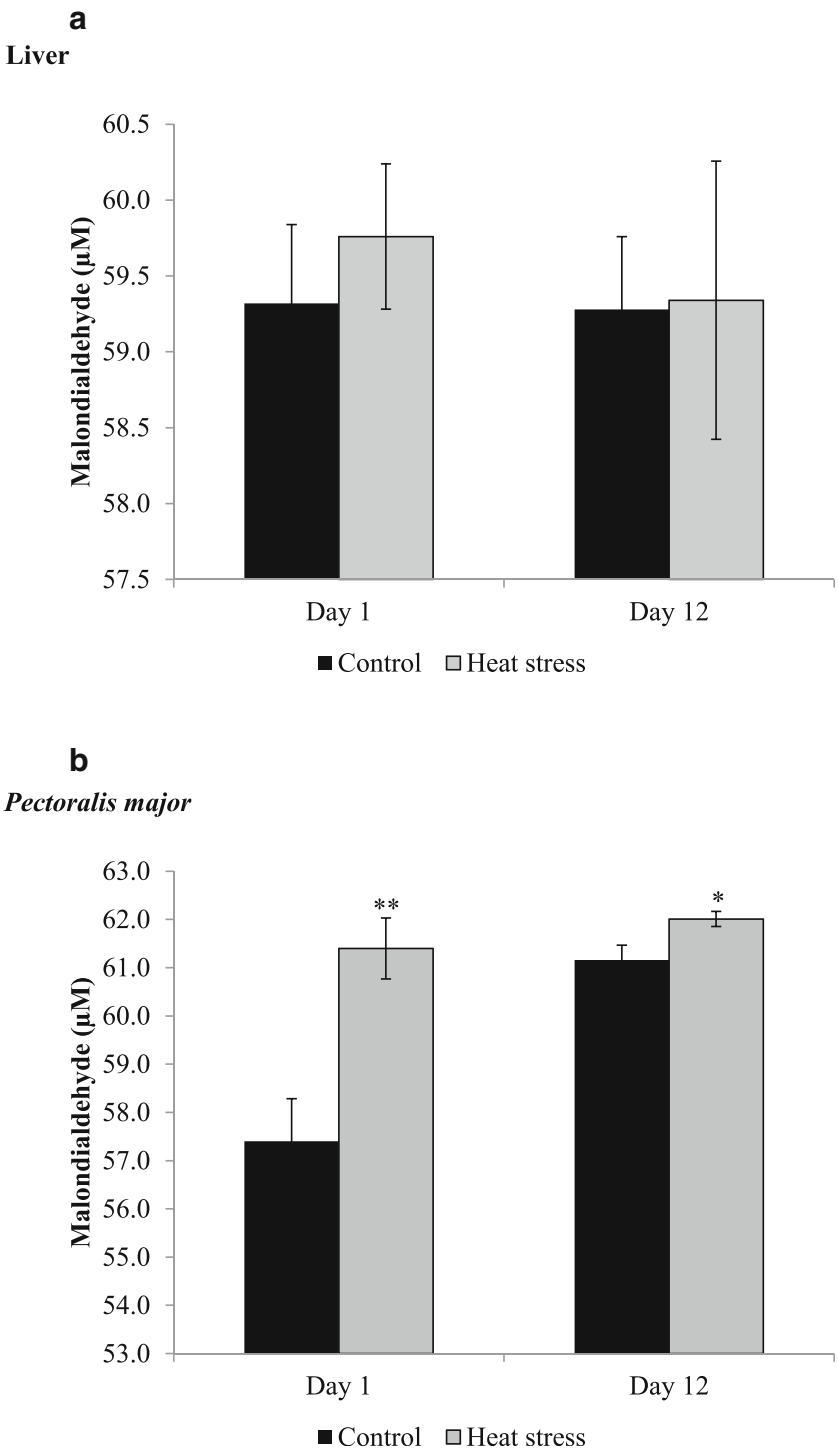

Fig. 11 Effect of heat stress on malondialdehyde activity in the liver (**a**) and *Pectoralis major* (**b**) tissue of broiler (**$P < 0.01$ and *$P < 0.05$)

OHdG levels increased when chickens were subjected to chronic HS; however, no such increase was observed in the *P. major* muscle. Studies have demonstrated the direct relationship between increased hydroxyl radicals and DNA damage (Zhang et al. 2004; Kojima et al. 2010). The product of glucose oxidation when bound to proteins or ROS oxidation of glycated residues in proteins is irreversible and produces AGEs (Jenkins and Lyons 1997). AGEs have been shown to be associated with some diseases including diabetes mellitus and neurodegenerative diseases (Bucala and Cerami 1992; Ahmed 2005). During oxidative stress, there is rerouting of glucose metabolism via the pentose phosphate pathway in an

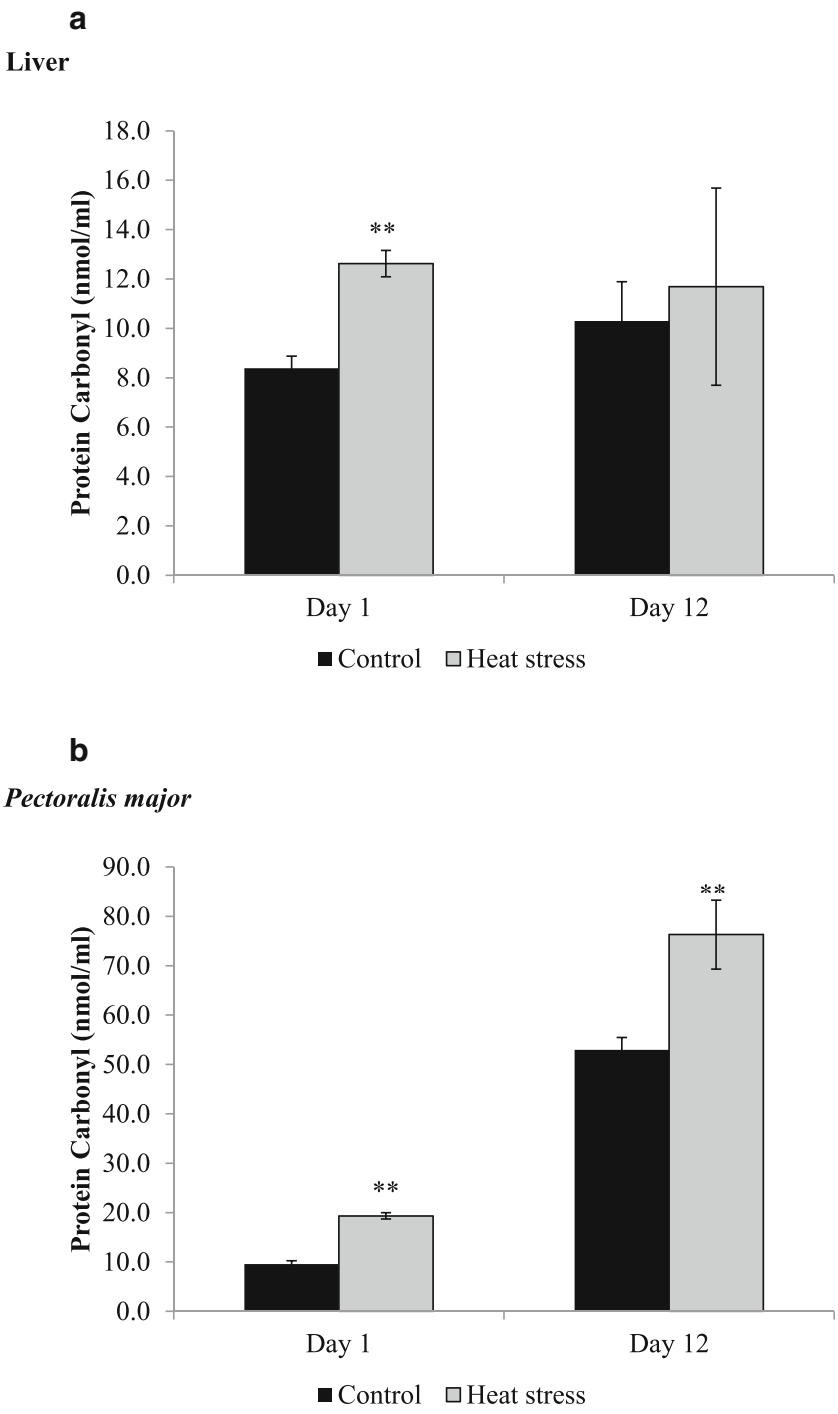

Fig. 12 Effect of heat stress on protein carbonyl activity in the liver (**a**) and *Pectoralis major* (**b**) tissue of broiler (**$P < 0.05$)

attempt to maintain redox balance and ROS clearance (Kuehe et al. 2015). Therefore, heat waves can lead to glycation which may render poultry vulnerable to other pathological diseases.

ROS can also damage the cellular membrane lipid bilayer arrangement and in the process may inactivate membrane-bound receptors and increase tissue permeability (Girotti 1985). The process generates relatively stable end products such as MDA and isoprostanes which can be measured in plasma or tissues as markers of oxidative stress. The stable end products are also capable of inactivating many cellular proteins by forming protein cross-linkages (Siu and Draper 1982). We observed a significant increase in MDA levels in the *P. major* muscle when chickens were subjected to both acute and chronic heat stress. The MDA levels in the liver did not change under heat stress; however, Ramnath et al. (2008) reported that plasma MDA levels were elevated when chickens were subjected to heat stress. Lipid peroxidation in the *P. major* muscle may affect meat quality, as Zhang et al.

(2011) showed that oxidative stress in live chickens accelerated post-mortem glycolysis and increased drip loss in broiler breast muscles.

Oxidative stress also modifies protein function resulting in protein carbonylation and protein nitration which are used as biomarkers (Dalle-Donne et al. 2003, 2006; Sultana et al. 2013). Protein oxidation leads to increased vulnerability to proteolysis by degradation of specific proteases (Kelly and Mudway 2003). PCO levels in the liver increased only under acute heat stress but not under chronic heat stress; however, the *P. major* muscle exhibited high levels of protein oxidation when the chickens were exposed to both acute and chronic heat stress. It has been demonstrated that methionine and cysteine residues in proteins are the most susceptible to oxidation (Dean et al. 1985; Levine et al. 2000; Zhang et al. 2013), and oxidation of methionine causes conformation changes, protein unfolding, and degradation (Lyras et al. 1997). Recently, Habashy et al. (2017a, b) showed that the amount of cysteine per unit of growth was the highest among all amino acids under heat stress further signifying the relative importance of cysteine in chickens under heat stress. Protein oxidation in the muscle increases the muscles' susceptibility to proteolysis and this could be a major factor contributing towards reduced growth under heat stress. Protein oxidation in the muscle could also change the muscle pH and that could affect meat quality.

Conclusion

Heat stress causes oxidative stress in chickens and subsequently elicits changes in the oxidant/antioxidant cellular mechanisms to respond to the oxidative stress. The principal redox pathways including SOD, CAT, and GSH-GPx respond sometimes differently in the liver and the *P. major* muscle according the tissue's need to detoxify ROS or tolerance for ROS. Biomarker changes show that heat stress causes carbohydrate and DNA oxidation in the liver. Lipid peroxidation and protein oxidation significantly occur in the *P. major* muscle of chickens under heat stress.

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