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Electrolyzed reduced water could regulate muscular energy producing ability of heat-exposed broiler chickens

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Abstract

We have previously shown that alleviation in ROS-induced oxidative damage to skeletal muscle and improvement in production efficiencies are observed in broiler chickens supplied with electrolyzed-reduced water when exposed to 5 days-long chronic heat exposure. In this study, we investigated whether this reduced water may also improve those parameters in broiler chickens exposed to 14 days-long chronic heat exposure. Broiler chickens (*Gallus gallus*) were fed either a control diet with tape water or control diet supplied with electrolyzed-reduced water for 14 d after hatch, and then exposed either to heat stress (34ºC for 14 days), or kept at a thermoneutral temperature (24ºC). Skeletal muscle substrate metabolism (3HADH and CS activities), adenine nucleotides concentration, enzymatic scavenging systems were studied. We confirmed that heat-stressed broilers decreased weight gain and feed consumption compared to control broilers. In contrast, heat-exposed broilers supplied with electrolyzed-reduced water significantly and definitely improved performance when compared to heat-stressed control broilers. On exposure to long-term heat treatments, broiler supplied with reduced water showed higher CS activity, indicating that the reduced water could improve energy-producing ability via modulating TCA cycle. We also found that the reduced water could definitely improve the total adenine nucleotide (TAN), adenylated energy charge (AEC), and ATP to ADP ratios under such conditions. On the other hand, broiler chickens supplied with reduced water kept relatively constant the SOD, catalase, and GPx activities, implying that reduced water could support the enzymatic scavenging systems. Taken together, these results suggest that broiler chickens supplied with electrolyzed-reduced water could improve energy producing ability via modulating TCA-cycle enzyme, and lead to improving performance.

Introduction

In chickens, either ‘acute’ or ‘chronic’ heat stress induces elevated body temperature due to have difficulty in balancing heat production and dissipation. Moreover, this elevated body temperature in both stressed-conditions decreases feed consumption, weight gain (Mujahid et al., 2005; Lu et al., 2007; Azad et al., 2010 a, b, c), and increases mortality (Geraert et al., 1996). Besides these, hyperthermia-induced stimulated reactive oxygen species (ROS) formation was observed ‘in vitro’ conditions in skeletal muscle of broiler chickens (Feng et al., 2008). Our group has provided direct evidence that acute heat stress stimulated mitochondrial ROS generation, and was also speculated that this increase possibly decreased the production performance of the birds (Mujahid et al., 2009). More recently, a time course study with chronic heat stress protocol revealed that mitochondrial ROS generation was definitely increased from 3 to 9 days after heat exposure, whereas the reduction in body weights are started after 3 days of heat exposure (Azad et al., 2010 b). It appears that both heat-stressed conditions induce enhanced ROS production, and depressed production performance. Therefore, one could speculate that reactive oxygen species (ROS) may be involved in the growth retardation process of heat-stressed broiler chickens. Thus it can be hypothesized that antioxidant that possess not only ROS-scavenging ability but also energy producing ability could promote production performance by reducing ROS production. With that point of view, it was already proved the characteristics of electrolyzed-reduced water as a potent antioxidant which could effectively scavenge hydrogen peroxide, superoxide and hydroxyal radical, and protect DNA, RNA, proteins, cells, and tissues under strong oxidative stress conditions (Shirahata et al., 1997; Lee et al., 2006; Tsai et al., 2009a; Park et al., 2009; Azad et al., 2021) and the results of Kajiyama’s study also suggest that this water could improve lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance. Moreover, various biological effects of electrolyzed-reduced water have been reported such as anti-diabetic effect (Kim and Kim, 2006; Jin et al., 2006), growth-stimulating effect of fetus (Watanabe, 1995), and growth-stimulating effect of anaerobic microflora in the human intestine (Vorobjeva, 2005). Importantly, our group has recently found that electrolyzed-reduced water could attenuate skeletal muscle ROS production and oxidative damage in chickens exposed to 5 days-long constant 34ºC heat treatments (Azad et al., 2013). Furthermore, we observed that this water could improve production performance, which may possibly be due to increased metabolic oxidation capacity. It

is conceivable that this reduced water may also improve those parameters in broiler chickens exposed to 14 days-long chronic heat exposure. To confirm this points, broiler chickens (*Gallus gallus*) were fed either a control diet with tape water or control diet supplied with electrolyzed-reduced water for 14 d after hatch, and then exposed either to heat stress (34ºC for 14 days), or kept at a thermoneutral temperature (24ºC). In the present study, therefore, we determined i) mitochondrial substrate oxidation enzymes activities (3HADH and CS), and adenine nucleotides concentration for the clarification of metabolic characteristics; we also studied ii) the activities of enzymatic scavengers such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) to determine the antioxidant effect of this water in the skeletal muscle tissue of chronically-heat-exposed broilers.

Materials And Methods

Birds and experimental design

Meat-type chicks (Ross) (*Gallus gallus*) were obtained from a commercial hatchery (Economic Federation of Agricultural Cooperatives hatchery, Iwate, Japan) at 1 d of age and were housed in electrically-heated batteries under continuous light for 10 days and provided with *ad libitum* access to either tape or electrolyzed-reduced water and commercial starter meat-type chick diet (crude protein, 23%; metabolizable energy content, 3150 kcal/kg). After a 3-d adaptation period, 24 broiler chickens were divided at uniform body weights into four groups. One of the two groups was then exposed to either a constant heat stress 34ºC for 14 d, while the other group was maintained at 24ºC (humidity 55 $\pm$ 5%). Birds were provided with *ad libitum* access to either tape or electrolyzed-reduced water and control diets during the treatments and then sacrificed by decapitation, and pectoralis superficialis muscles were rapidly excised. To study the mitochondrial substrate metabolism enzyme activities, antioxidant enzyme activities, and adenine nucleotides concentration, muscles were frozen, powdered in liquid nitrogen, and stored at -80°C until required for analysis. All experiments were performed in accordance with institutional guidelines concerning animal use and efforts were made to minimize pain or discomfort of the animals.

Measurement of 3HADH and CS activity

3-hydroxylacyl CoA dehydrogenase (3HADH; EC 1.1.1.35) and citrate synthase (CS; EC 4.1.3.7) activities were measured according to the methods of Bradshaw et al. (1975) and Srere (1969), respectively. Briefly, pectoralis superficialis muscle was used for the measurement of 3HADH and CS activity. For 3HADH, tissues were homogenized in buffer [175 mMKCl, 10 mM glutathione (reduced form), 2 mM EDTA, and 10% Triton X-100, pH 7.0], briefly centrifuged at 3000 rpm for 10 min at 4ºC, and the supernatants then collected. In brief, 5 µl of supernatants were mixed with 967 µl of reaction buffer (TEA.EDTA, pH 7.4), 20 µl of NADH. After mixing, the mixture solution was incubated in a water bath for 3 min and then 8 µl of acetoacetyl CoA was added to the mixture solution and 3HADH activity measured spectrophotometrically (U-3010, Hitachi, Japan) using the following conditions: temperature 30ºC, absorbance 340 nm, and duration of measurement 3 min. 3HADH activity was expressed as nmol/min/g wet tissue. For CS activity, tissues were homogenized in buffer [250 mM sucrose, 20 mMTris, 1 mM EGTA, and 10% Triton X-100, pH 7.4], briefly centrifuged at 3000 rpm for 10 min at 4ºC, and the supernatants collected. In brief, 5 µl of tissue supernatants were mixed with 2 ml of reaction buffer (0.1M Tris-HCl, pH 8.0), 20 µl of 10M DTNB (freshly-prepared), 20 µl of 5 mM acetyl CoA, 10 µl of 50 mM oxaloacetate and CS activity measured spectrophotometrically (U-3010, Hitachi, Japan) using the following conditions: temperature 25ºC, absorbance 412 nm, and duration of measurement 6 min. CS activity was expressed as nmol/min/g wet tissue.

Measurement of superoxide dismutase (SOD) activity

Superoxide dismutase activity (SOD; EC 1.15.1.1) in the pectoralis muscle of broiler chickens was measured using a commercially-available kit from Cayman Chemical Company (Catalog Number 706002, Ann Arbor MI, USA). Briefly, SOD activity was quantified using a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. At the time of analysis, approximately 100 mg of pectoralis muscle tissue was homogenized in 20 mM HEPES buffer containing 1 mM EGTA, 210 mMmanitol and 70 mM sucrose. Homogenization was performed using a handy micro homogenizer (NS-310E) for 45 seconds with low speed set to 6 per second. After homogenization, homogenates were centrifuged at 1500×g for 5 min at 4ºC. The supernatant was then removed. Prior to measurement of SOD activity, tissue homogenates were diluted with sample buffer (diluted) 20 times to produce absorbances within the linear range of the standard curve. Analysis of the tissue homogenates was carried out according to the manufacturer's instructions in 96-well format. Standard curves were run on each plate and were generated by measuring the optical density of seven samples with SOD activity ranging from 0-0.25 U/ml. The absorbance of the sample and standard wells were monitored at 450 nm using a plate reader (Bio-rad model 680, Japan Bio-rad laboratories Co. Ltd. Tokyo, Japan). SOD activity was expressed as U/g wet tissue. One unit of SOD is defined as the amount of enzyme needed to cause 50% dismutation of the superoxide radicals.

Determination of glutathione peroxidase (GPx) activity

Glutathione peroxidase activity (GPx; EC 1.11.1.9) in the pectoralis muscle of broiler chickens was determined by using a kit available from Cayman Chemical Company (Catalog Number 703102, Ann Arbor MI, USA). The GPx assay kit measures GPx activity by way of a coupled reaction with glutathione reductase. Oxidized glutathione is produced during reduction of an organic hydroperoxide by GPx, and is recycled to its reduced state by glutathione reductase and NADPH. The oxidation of NADPH to $NADP^+$ causes a decrease in the absorbance of the samples at 340 nm is directly proportional to the GPx activity in the sample. At the time of analysis, approximately 100 mg of pectoralis muscle tissue was homogenized in 50 mMTris-HCL buffer containing 5 mM EDTA, 1 mM DTT. Homogenization was carried out as described above. After homogenization, homogenates were centrifuged at 10,000×g for 15 min at 4ºC. The supernatant was then removed. Prior to measurement of GPx activity, tissue homogenates were diluted with sample buffer (diluted) 25 times to produce absorbances within the linear range of the assay. Analysis of the tissue homogenates was carried out according to the manufacturer's instructions in 96-well format. The absorbance of the sample and control wells was monitored at 340 nm using a plate reader (Labsystems Multiskan MS-UV, Finland) at five time points spanning 5 min. GPx activity was expressed as nmol/min/g wet tissue.

Quantification of catalase (CAT) activity

Catalase activity (CAT; EC 1.11.1.6) in the pectoralis muscle of broiler chickens was quantified using a commercially-available kit from Cayman Chemical Company (Catalog Number 707002, Ann Arbor MI, USA). This method is based on the reaction of the CAT enzyme with methanol in the presence of an optimal concentration of H_2O_2. The formaldehyde produced was measured spectrophotometrically at 540 nm using 4-amino-3hydrazino-5-mercapto-1, 2, 4-triazole (Purpald) as the chromozen. Purpald specifically forms a bicyclic heterocycle with aldehydes, which changes from colorless to purple following oxidation. At the time of analysis, approximately 100 mg of pectoralis muscle tissue was homogenized in 50 mM Potassium Phosphate buffer containing 1 mM EDTA. Homogenization was carried out as described above. After homogenization, homogenates were centrifuged at 10,000×g for 15 min at 4ºC. The supernatant was then removed. Prior to measurement of CAT activity, tissue homogenates were diluted with sample buffer (diluted) 50 times to produce absorbances within the linear range of the standard curve. Analysis of the tissue homogenates was carried out according to the manufacturer's instructions in 96-well format. Standard curves were run on each plate and were generated by measuring the optical density of seven standards. The absorbance of the sample and standard wells were monitored at 540 nm using a plate reader (Bio-rad model 680, Japan Bio-rad laboratories Co. Ltd. Tokyo, Japan). CAT activity was expressed as U/g wet tissue.

HPLC analysis of adenine nucleotide contents in skeletal muscle

Pectoralis muscle tissue was used for the quantification of ATP, ADP and AMP concentrations. Briefly, frozen tissues were kept in liquid nitrogen. About 50~100 mg of tissue were weighed, to which 1 ml of ice-cold perchloric acid was added. Then, the mixture was homogenized for about 45 seconds on iced-bath with a homogenizer (handy micro homogenizer, NS-310E). The homogenates were centrifuged at 3000×g for 10 min at 4ºC. The supernatants were neutralized with 200 µl of neutralizing agent (2.0 mol/L K_2CO_3) added to 0.5 ml of the supernatant, kept on ice for 10 min, and then centrifuged at 3000×g 4ºC for 10 min. Supernatants were stored at -80ºC until HPLC assay was performed. The HPLC assay system consisted of a binary pump, degasser and column oven (Shimadzu, LC-10AD vp, DGU-12A and CTO-10AC vp, respectively) and a UV detector (JASCO, UV-970). A Waters XBridge C18 (5µm, 4.6mm×150mm) chromatographic column was used in this assay. The mobile phase (pH 7.0) was prepared by a mixture of 50 mmol/L K_2HPO_4-KH_2PO_4 buffer solution and methanol (77:23, v/v), and then filtered through a 0.45µm filter membrane and degassed 15 min prior to use. The detection wavelength, range, and flow rate was 260 nm, 1.28, and 1.0 ml/min, respectively. The column temperature was 35ºC. The frozen supernatants were filtered through a 0.20µm filter membrane after thawed at room temperature, and final injection volume was 10 µl. Peaks were identified by their retention times by using co-chromatography standards. ATP, ADP and AMP standards (2 mg) were each dissolved in 10 ml of deionized water to obtain ATP, ADP and AMP standard stock solutions at 200 µg/ml. Standard solutions were made by diluting stock solutions with mobile phase as a solvent to a series of concentration 2 µg/ml, 4 µg/ml, 5 µg/ml, 8 µg/ml, 10 µg/ml, and 12 µg/ml, then performed HPLC assay, the peak areas were detected. The standard curve was constructed by plotting peak area vs concentration. Linear curves were obtained. ATP, ADP and AMP concentration in the test sample solution were calculated from standard curves according to corresponding peak areas. ATP, ADP and AMP content in each sample were calculated according to their concentration in the test sample solution, sample wet weight and dilution coefficient. Total Adenine Nucleotide (TAN) and Adenylate Energy Charge (AEC) were calculated according to the following equation. TAN= (ATP+ADP+AMP), AEC= (ATP+0.5ADP)/(ATP+ADP+AMP).

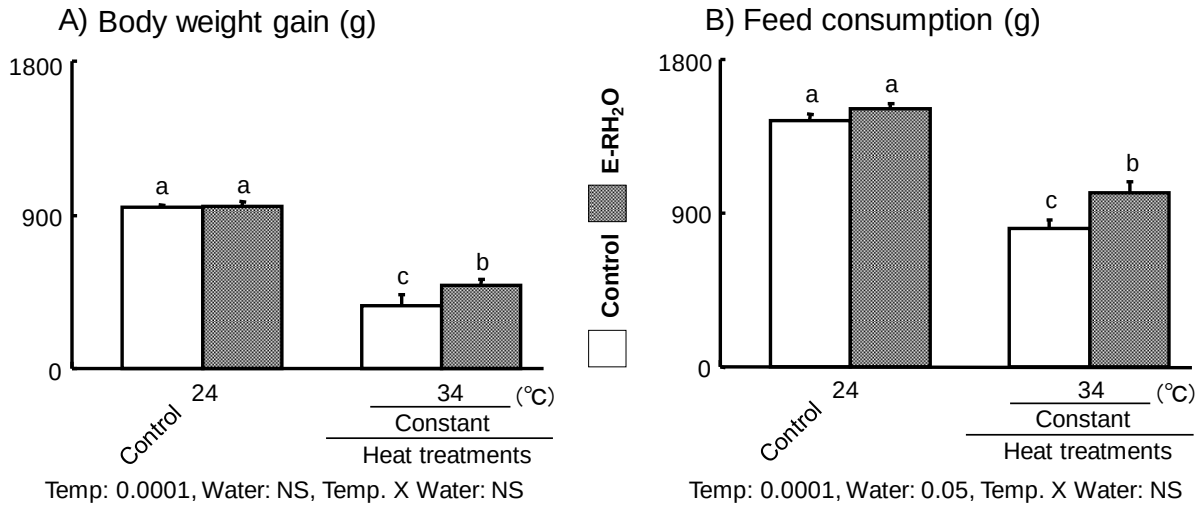
Statistical analysis

Data were analyzed using the statistical analysis system (SAS, 1985). Data were first analyzed by a general linear model analysis of 2-way anova procedure and the means were compared using Duncan's least significance multiple-range test. All data are expressed in the form of mean ± standard error (SE). Differences were considered significant for values of $P < 0.05$.

Results

Growth performance

As shown in Fig. 1A, B, heat-stressed broilers showed a significant decrease in body weight gain and feed consumption compared to control broilers. In contrast, heat-exposed broilers supplied with electrolyzed-reduced water significantly and definitely improved performance when compared to heat-stressed control broilers. Broilers supplied with electrolyzed-reduced water showed no changes in these parameters under the conditions of thermoneutrol temperature.



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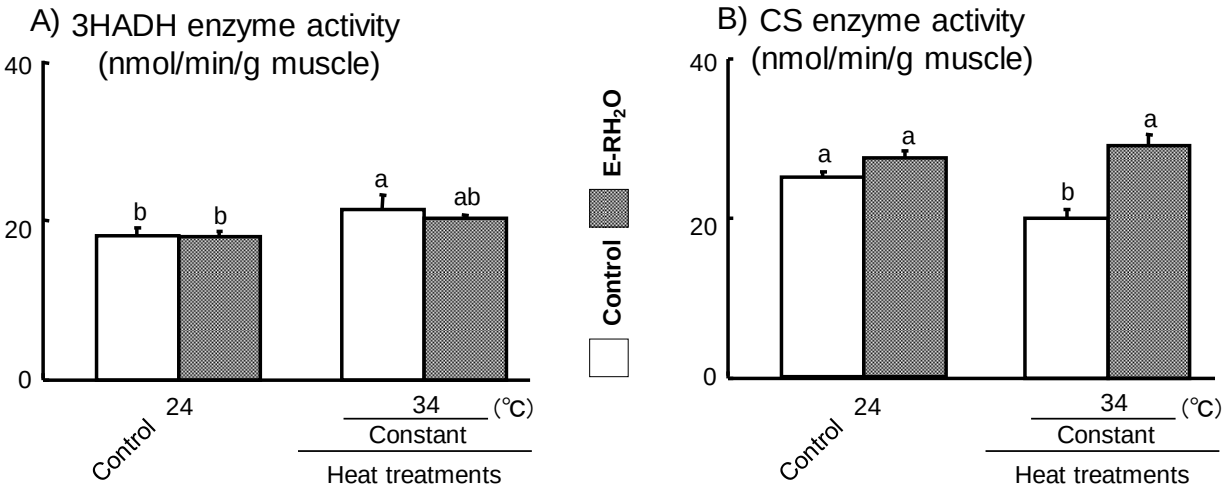
Metabolic oxidation capacity

As shown in Fig.2 A, B, heat-stressed broilers exhibited a significant increase in 3HADH activity compared to control broilers. In contrast, heat-exposed broilers supplied with electrolyzed-reduced water kept relatively constant 3HADH activity in both conditions. Heat-stressed broilers showed a significant decrease in CS activity compared to control broilers. On the other hand, heat-treated broilers supplied with this water significantly improved CS activity when compared with heat-stressed control broilers. Such improvement in CS activity was not observed in thermoneutral conditions.



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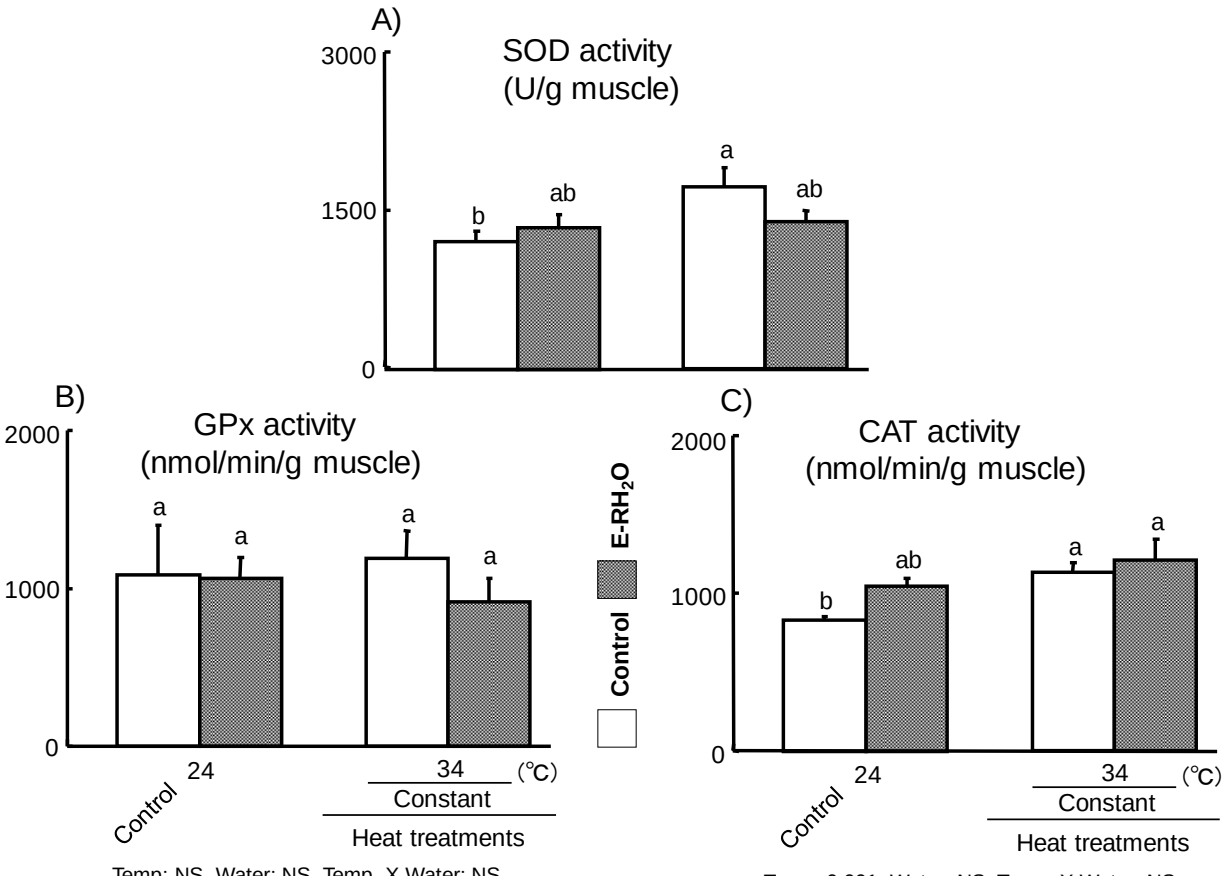
Temp: 0.01, Water: 0.0001, Temp. X Water: NS

Enzymatic scavenging systems

Fig. 3 shows the effects of temperature and water on antioxidant enzymes activity in the pectoralis muscle of broiler chickens. Heat-exposed broilers showed a significant increase in skeletal muscle SOD and CAT activities, and were kept relatively constant the activity of GPx compared to control broilers. On the other hand, broiler chickens supplied with electrolyzed-reduced water kept relatively constant the SOD, CAT, and GPx activities in both conditions.



Temp: NS, Water: NS, Temp. X Water: NS

Temp: 0.001, Water: NS, Temp. X Water: NS

Muscle metabolites concentration

Table 1 shows the effect of temperature and water on adenine nucleotides concentration in the pectoralis muscle of broiler chickens. Heat-stressed broilers did not show any changes in the concentrations of AMP, ADP, or ATP compared to control broilers. On the other hand, broiler chickens supplied with electrolyzed-reduced water kept these metabolites concentration relatively lower than control groups in both conditions. Broiler chickens supplied with electrolyzed-reduced water showed comparatively lower the concentrations of the total adenine nucleotide pool (ATP+ADP+AMP) under the thermoneutral condition, while this pool was tended to increase in heat-stressed chickens supplied with electrolyzed-reduced water when compared with heat-stressed control broilers. Adenylate Energy Charge (AEC) and ATP/ADP ratios as indicators of the metabolic status in chickens, was definitely improved in heat-exposed broiler chickens supplied with electrolyzed-reduced water compared to heat-stressed control broilers, whereas no change was observed with these parameters under thermoneutral-exposed broiler chickens.

		Temperature		P-value		
Muscle variable	Water	Thermoneutral[1]	Heat-exposed[2]	Temp	Water	Temp × Water
AMP (µmol/g wet tissue)	Control	0.18 ± 0.02^{a}	0.19 ± 0.02^{a}	NS	NS	NS
	E-RH_2O	0.17 ± 0.01^{a}	0.17 ± 0.02^{a}			
ADP (µmol/g wet tissue)	Control	1.6 ± 0.14^{a}	1.4 ± 0.14^{ab}	NS	NS	NS
	E-RH_2O	1.1 ± 0.14^{b}	1.3 ± 0.05^{ab}			
ATP (µmol/g wet tissue)	Control	6.5 ± 0.68^{a}	5.6 ± 0.40^{a}	NS	NS	*
	E-RH_2O	4.8 ± 0.73^{a}	6.6 ± 0.48^{a}			
TAN (µmol/g wet tissue)	Control	8.2 ± 0.76^{a}	7.2 ± 0.51^{ab}	NS	NS	*
	E-RH_2O	6.0 ± 0.87^{b}	8.0 ± 0.51^{a}			
AEC (µmol/g wet tissue)	Control	0.88 ± 0.01^{ab}	0.88 ± 0.01^{ab}	NS	NS	NS
	E-RH_2O	0.85 ± 0.01^{b}	0.90 ± 0.01^{a}			
ATP to ADP ratio	Control	4.2 ± 0.37^{a}	4.2 ± 0.29^{ab}	NS	NS	NS
	E-RH_2O	4.2 ± 0.26^{a}	5.6 ± 0.29^{a}			

AMP: Adenosine monophosphate, ADP: Adenosine diphosphate, ATP: Adenosine triphosphate, TAN: Total adenylated nucleotide pool, AEC: Adenylate energy charge (ATP+1/2ADP)/ (ATP+ADP+AMP). [1]Maintained at 24ºC, [2]Maintained at 34ºC. Means within rows with no common superscript differ significantly ($P<0.05$). *$P<0.05$; NS = not significant.

Discussion

Heat stress induces mitochondrial ROS generation, causing oxidative damage to mitochondrial lipids and proteins (Mujahid et al., 2007b; Azad et al., 2010b). More recently we found that chronic heat stress impairs anti-oxidation capacity, and enhances lipid peroxidation in the skeletal muscle of broiler chickens (Azad et al., 2010a).This cellular hazardness further leads to suppressing growth performance, indicating that active oxygen species could mediate in the growth retardation process of stressed birds. This active oxygen species may be reversed by ERW supplementation, because this water contains large amount of active hydrogen that was already proved as an effective scavenger for H_2O_2, superoxide, and hydroxyl radical (Shirahata et al., 1997; Tsai et al., 2009a; Azad et al., 2013; 2021). Several lines of evidence also indicate that ERW had the ability of a scavenger free radical, such as H_2O_2, the hydroxyl radical, and the superoxide radical, to protect DNA, RNA, proteins, cells, and tissues against strong oxidative stress (Shirahata et al., 1997; Huang et al., 2003; Lee et al., 2006). Therefore, we considered that ERW is useful in recovering heat-induced oxidative damage in the skeletal muscle by improving growth performance. In the present study, the capability of ERW to protect heat-induced performance inhibition and oxidative in broiler skeletal muscle was first investigated. In the case of CPW (cartridge purified water), no differences in the levels of MDA in the skeletal muscle, liver or plasma was observed at 34ºC-exposed birds (data not shown). This is in contrast with the results of our previous report (unpublished results), where we demonstrated a significant increase in the levels of MDA in the plasma and tissues. The reason for this difference could be the CPW used in our investigation instead of tape water. Concerning the mechanism responsible for the persistence in skeletal muscle MDA production during CPW supplementation, there are various possibilities. A simple possible reason may be an increase in the levels of the scavenger enzyme activities responsible for ROS generation. In fact, in the present study, SOD and CAT levels were significantly elevated without changes in the GPx level by CPW supplement to the heat stressed birds, suggesting that it has the ability to restore these enzymes' activities in heat-exposed broiler skeletal muscle. Mitochondrial fatty acid metabolism could also be another possible reason for the persistence in skeletal muscle MDA levels. In our previous study, birds exposed to 34ºC not only produce high MDA levels, but also produce less fatty acids (as shown in decreased 3HADH activity and CPT1 mRNA levels; data not shown) for transportation into the mitochondria. Contrary to previous work, 3HADH activities were significantly increased but were not changed in the levels of CPT1 mRNA in the present study, proposing that mitochondrial fatty acids were regulated by the degree of enhancement in MDA levels of the skeletal muscle at 34ºC condition. Taking account into the above two possible reasons, we could say CPW had some beneficiary effects to reduce oxidative damage under heat-exposed condition. Electrolyzed hydrogen-saturated water could inhibit lipid peroxidation in the rat liver (Yanagihara et al., 2005). More recently it has been reported by Nagata et al. (2009) that hydrogen

rich water could suppress chronic restraint stress-induced oxidative damage in the mice brain. In the present study, ERW in the heat stress condition exhibited a non-significant decrease in both the skeletal muscle and liver MDA levels, and kept a significant reduction in the plasma MDA level. Although high ERW in the thermoneutral condition significantly reduced liver and plasma MDA levels, the amplitude of this reduction was relatively smaller in the skeletal muscle than that for liver and plasma MDA levels. These results suggestthat ERW supplementation can partially inhibit the increase of MDA level in the skeletal muscle under both the thermoneutral and heat-exposed conditions (data not shown). Superoxide dismutase (SOD) is an effective defense enzyme that catalyses the dismutation of superoxide anions into hydrogen peroxide (H_2O_2) (Reiter et al., 2000). Catalase (CAT) metabolizes the excess of H_2O_2 producing O_2 + H_2O, thereby decreasing the intracellular redox status. Glutathione peroxidase (GPx) is an important enzyme in the detoxification of xenobiotics in the liver and converted the reduction of H_2O_2 and hydroperoxides to non-toxic products.Shirahata et al. (1997) were the first to report that ERW exhibits both SOD-like and catalase-like activities. They also found that the SOD-like activity of ERW is stable at 4ºC for over a month and is not lost even after neutralization, repeated freezing and melting, deflation with sonication, vigorous mixing, boiling, repeated filtration, or closed autoclaving. More recently it has been demonstrated by Tsai et al. (2009b) that ERW supplement significantly ameliorated the Carbon tetrachloride (CCl_4)-induced suppression of the activities of SOD, catalase, and GPx in the mice liver. In the present study, ERW in the heat stress condition showed a non-significant increase in both the CAT and GPx activities, and kept a slight decrease in the SOD activity. On the contrary, this water in the thermoneutral condition showed a trend to increase in both the SOD and CAT activities, and kept GPx at control levels. These results suggest that the response of high ERW against ROS via initializing antioxidant enzymes activity depends on the temperature protocol. Compared to the control water group, a significant decrease in body weight gain, feed consumption, and feed efficiency were seen in the heat-stressed broiler with the same water treatment. These results are in consistent with our previous work and others who reported that heat stress decreased performance terms. Oda et al. (1999) reported that ERW stimulates glucose uptake in muscle cells and adipocytes, and more recently it has been reported by Kajiyama et al. (2008) that hydrogen-rich water supplementation improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance. Tsai et al. (2009b) proposed that ERW had the ability to protect liver against CCl_4-induced oxidative damage in mice as reflected by decrease in liver weight, expecting that rate of feed nutrients utilization may improve under heat stress conditions, thereby improving feed intake and growth rate. Indeed, we found a significant increase in feed intake and body weight gain in broiler reared under heat stress with ERW supplement. In situations which feed consumption is increased, the feed forward enzyme like citrate synthase may increase, which may lead to TCA cycle modulation for the increase energy production. In this way, ERW significantly improved CS activity and exerted positive effects on metabolites components, namely total adenine nucleotide pool (TAN), adenylated energy charge (AEC), and ATP/ADP ratios in broiler reared under heat stress (34ºC; Table 1 and Figure 2).

Conclusions

These results indicate that chickens with ERW supplementation had a higher metabolic activity under long-term heat stress condition. Interestingly, we did not find any significant effects of ERW on mRNA levels (data not shown) of the mitochondrial fatty acid transport and oxidation enzymes in chickens reared under both the thermoneutral and heat stress conditions.

Conflicts of Interest

The authors declare that there are no potential conflicts of interests.

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