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Research Article

Comparative study of certain antioxidants - electrolyzed reduced water, tocotrienol and vitamin E on heat-induced oxidative damage and performance in broilers

MAK Azad[1], M Kikusato[2], I Zulkifli[4], MM Rahman[1], MS Ali[3], MA Hashem[1] and M Toyomizu[2*]

[1]Department of Animal Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]Animal Nutrition, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amamiyamachi, Aoba-ku, Sendai 981-8555, Japan
[3]Department of Poultry Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
[4]Department of Animal Science, Institute of Tropical Agriculture, University Putra Malaysia, UPM Serdang, 43400, Selangor, Malaysia

***Corresponding author:**
Professor Dr. Masaaki Toyomizu
E-mail: toyomizu@bios.tohoku.ac.jp

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Abstract

This study was designed to examine the anti-oxidative effect of electrolyzed reduced water, tocotrienol and vitamin E on heat-induced oxidative damage and performance in an experimental model in broilers. On day 12, broiler chickens were subjected to one of the following dietary groups; (i) basal diet and untreated drinking water (control), (ii) basal diet and electrolyzed reduced drinking water (ERW), (iii) basal diet supplemented with 2% rice bran scum oil (as a source of tocotreinol) and untreated drinking water (TOCO), and (iv) basal diet supplemented with vitamin E at 50 mg/kg and untreated drinking water (VITE). On day 14, chickens were exposed to either 34°C continuously for a period of 14 days, or maintained at 24°C on the same diet. Heat-exposed birds consumed significantly less feed resulting in lower weight gain and feed efficiency compared with birds kept at 24°C. Skeletal muscle and liver MDA levels were significantly increased in heat-exposed control birds. The heat-exposed ERW chicks showed significantly improved growth performance and lower levels of MDA contents in tissues than heat-stressed control broilers. Following heat exposure, TOCO and VITE chicks did not exhibit improved performance, while those chicks significantly reduced oxidative damage to the various organs. The results demonstrate that electrolyzed reduced water, tocotrienol and vitamin E effectively protect heat-induced oxidative damage in broilers but they do not improve growth performance except electrolyzed reduced water treatment.

Introduction

Antioxidants are classified as synthetic or natural according to their origin. Synthetic antioxidants are derived from phenolic (i.e. BHA, BHT, TBHQ, dodecyl, propyl, and octyl gallate) or non-phenolic (ETOX) structures which have been widely used as food preservatives because of their effectiveness and relatively low cost. On the other hand, natural antioxidants (i.e. tocopherol, retinol, selenium, ascorbate, and other carotenoid forms) are commonly used as a practice to alleviate any stress effect in humans and animals (Bithi et al., 2020; Disha et al., 2020; Saba et al., 2018; Sarker et al., 2021). Interestingly, both type of antioxidants act as a scavenger to remove free radicals (FR) initiators and propagators.

The relationship between heat stress and the increased production of FR and reactive oxygen species (ROS) in birds is well documented (Altan et al., 2003; Mujahid et al., 2006; Azad et al., 2010b). These agents react destructively with cellular molecules (i.e. proteins, DNA, lipids) to cause cell damage. Heat-induced ROS formation could therefore be responsible for growth retardation in chickens. Though vitamin C, vitamin E, melatonin, and polyphenols were tested FR scavenger, they scavenged ROS effectively but could not improve growth performance of chickens exposed to cyclic heat stress (Sahin et al., 2003, 2004; Gharib et al., 2008; Wang et al., 2006). There is still no single dietary antioxidant available which can scavenge ROS effectively and improve growth parameters of broilers under constant heat stress conditions. Therefore, there is an increasing demand for new substances that possess scavenging properties for free radical, that are able to reduce ROS production and oxidative damage in tissues, and improve growth performance of birds under heat stress conditions, concomitantly.

Shirahata et al. (1997) were the first to report that electrolyzed reduced water contains large amounts of active hydrogen. Active hydrogen in ERW may be an ideal scavenger against ROS because it does not produce oxidized molecules after reduction like other organic antioxidants (i.e. vitamin C, vitamin E, and polyphenols). Moreover, the water exhibits high pH (10.5), low dissolved oxygen (5.85 mg/l) and significant negative redox potential (-151 mV), exhibits both SOD- and catalase-like activities. The authors also found that the properties of this water was stable at 4ºC for over a month and was not lost even after neutralization, repeated freezing and melting, deflation with sonication, vigorous mixing, boiling, repeated filtration, or closed autoclaving. Recently, Azad et al. (2013) reported that ERW could lessen ROS-induced oxidative damage and improve growth performance in short-term heat-exposed broilers. A number of studies have also demonstrated various biological effects of electrolyzed reduced water 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).

Tocotrienol (T_3, unsaturated vitamin E) is limited to certain feed sources (i.e. rice bran and palm oil) (Sundram et al., 2002; Sookwong et al., 2007). Tocotrienols are analogues of tocopherols, their chemical structures differ only in the degree of unsaturation of the side chain; tocotrienols present three isolated double bonds while tocopherols have a saturated side chain. It is generally believed that α-tocopherol is an extremely efficient inhibitor of free radical chain reactions (Qureshi et al., 2000). However, recent results indicated that α-tocotrienol is at least three times more efficient than α-tocopherol as a scavenger of peroxyl radicals in liposome membranes systems (Packer, 1995). Yamaoka et al. (1991) compared the antioxidative activities of tocopherols and tocotrienols and concluded that addition of tocotrienols to a liposome solution provided greater protection against lipid oxidation than that of tocopherols. Serbinova et al. (1991) reported that α-tocotrienol exhibited greater antioxidant activity than α-tocopherol against lipid peroxidation in rat liver microsomal membranes. We could, therefore, infer that dietary tocotrienols may provide good protection against lipid peroxidation, resulting improved growth in broiler chickens under heat stress conditions.

α-tocopherol is the most active natural antioxidant used in animal feeds; it exhibits an antioxidant activity at lower concentrations and a prooxidant activity at higher concentrations (Chen et al., 1998; Franchini et al., 2002). Vitamin E is a major lipid-soluble antioxidant that is able to protect membrane-bound lipids from metmyoglobin/hydrogen peroxide-initiated oxidation (Buckley et al., 1989). Vitamin E is thought to reduce fatty acyl hydroxyperoxy radicals (ROO) to yield less reactive hydroperoxides (ROOH) (Perez et al., 2010). Vitamin E supplementation has improved the growth and feed utilization of birds and has substantially improved the stability of meat quality against oxidative deterioration (Guo et al., 2001; Srivan et al., 2010).

From the above reports, it appears that dietary supplementation of antioxidants may provide good protection against lipid peroxidation, resulting improved growth performance in broilers exposed to constant thermal exposure. In the present study, we therefore determined whether electrolyzed reduced water, tocotrienols and vitamin E can inhibit oxidative damage to cellular components, and subsequently improve the growth performance of broiler chickens when exposed to constant 2 wks heat stress. In this experiment, MDA content in pectoralis superficialis muscle, liver and plasma were measured for the clarification of ROS-induced oxidative damage following 14 days of heat exposure.

Materials and Methods

Animals and experimental design

One hundred and twenty day-old male commercial broiler chicks (Ross) were obtained from a commercial hatchery (Economic Federation of Agricultural Cooperatives hatchery, Iwate, Japan). Chicks were assigned in groups of 30 to four electrically-heated batteries and provided continuous lighting for 11 days. All the chicks were provided a basal diet (crude protein, 23%; metabolizable energy content, 3150 kcal/kg; energy level was fortified with 5% soybean oil) and untreated drinking water. Feed and water were provided *ad libitum*. On day 12, 96 birds were moved to individual cages in four environmentally controlled chambers (24 birds/chamber) for a 3-d adaptation period with experimental cages and subjected to one of the following dietary groups; (i) basal diet and untreated drinking water (control), (ii) basal diet and electrolyzed reduced drinking water (ERW), (iii) basal diet (energy level was fortified with 2% rice bran scum oil + 3% soybean oil) and untreated drinking water (TOCO), and, (iv) basal diet + vitamin E at 50 mg/kg and untreated drinking water (VITE). On day 14, 48 broiler chickens were divided at uniform body weights into eight groups. One of the four groups was then exposed to a constant 34ºC for a period of 14 days, while the other group was maintained at 24ºC. The relative humidity was kept as close as possible to 55%. Following 14 days of heat exposure, blood samples were collected from all birds in heparinized tubes. All birds were killed by decapitation, and the pectoralis superficialis muscle and liver (5g) were rapidly excised. Tissues were immediately frozen in liquid nitrogen and powdered. Meanwhile plasma was collected by centrifuging blood samples at 3000 rpm for 10 min. Plasma and tissues were stored at -80°C until required for further analysis. Individual body weight was recorded prior to and after heat exposure. Feed intake during heat exposure was measured. Body weight gain and feed efficiency for each chicken were calculated. Rectal temperatures for control and heat-treated chickens with dietary alterations were measured using a digital thermometer (Yokogawa, Singapore). All experiments were performed in accordance with institutional guidelines concerning animal use and efforts were made to minimize pain or discomfort of the animals.

ERW Apparatus

The apparatus for producing the ERW was a batch type electrolyzing device (Type TI-200, Nihon Trim Co., Osaka, Japan) equipped with platinum-coated titanium electrodes and consisting of two parts; one being for the purification of the water and the other was for its electrolysis. The apparatus maintained the pH of the water in the range from 8.10 to 10.1, ORP values from -160 mV to -607 mV, and water flow rate from 2.0 L/min to 3.4 L/min. The apparatus was connected to a water tap receiving town water, which was first purified and then electrolyzed to produce both the ERW used in the experiments. Plastic containers were used for water feeding, and the ERW was changed twice daily to keep the water conditions constant.

Pectoralis muscle, liver, and plasma MDA

Pectoralis superficialis muscle, liver and blood plasma were used for MDA measurements. Tissues or plasma were homogenized in buffer (100 mM KCl, 50 mM Tris-HCl, and 2 mM EGTA, pH 7.4), briefly centrifuged at 700 g and the supernatants collected. Lipid peroxidation was assayed colorimetrically as a 2-thiobarbituric acid reactive substance (TBARS; see Mujahid et al., 2007b). In brief, 400 µL tissue homogenate or plasma were mixed with 100 µL 8.1% SDS, 0.75 ml of 20% acetic acid (pH 3.5), 25 µL 0.8% butylated hydroxyl toluene (BHT), and 0.75 ml 0.8% 2-thiobarbituric acid (TBA). After vortexing, samples were incubated on ice for 60 min and heated at 95ºC for 60 min in a water-bath. After cooling, 2.5 ml of a mixture of n-butanol and pyridine (15:1, v/v) were added and the samples were mixed by vortexing. After centrifugation at 1000 g for 10 min, the organic layer was extracted and read spectrophotometrically at 532 nm. The TBARS content was expressed as nmol of malondialdehyde (MDA) per equivalent g wet tissue or ml plasma. The samples were analyzed within 1 week of storage at -80º C.

Statistical analysis

Data were analyzed by 2-way ANOVA using SAS (SAS institute, Carry, NC), with diet (control, ERW, TOCO, and VITE), temperature (unheated and heated), and the interaction between them as main effects. Comparisons among multiple means were made by Duncan's multiple range test. When interactions between main effects were significant, comparisons were made within each experimental variable. Differences were considered statistically significant at $P < 0.05$. The results in the tables are presented as the means and standard errors of the mean (SEM).

Results

Table 1 shows the growth performance results. Among the unheated birds, VITE chickens consumed significantly ($P < 0.05$) less feed than other groups. The feed intake of control, TOCO and ERW chickens were not significantly different. However, following heat exposure, ERW significantly ($P < 0.05$) consumed more feed than controls but not significantly different from those of TOCO and VITE. Diet had no significant effect on body weight gain of unheated chicks. On the contrary, the heated ERW chicks had significantly greater weight gains than controls but not significantly different from those of TOCO and VITE. While diet had no significant effect on feed efficiency of heated birds, the unheated VITE birds showed the best feed efficiency.

Table 1

Growth performance of broiler chickens fed the experimental diets.

Variables	Control	ERW	TOCO	VITE[1]
FI, g				
Unheated	1551.80 ± 29.81[a]	1548.27 ± 38.50[a]	1542.13 ± 36.82[a]	1382.17 ± 51.35[b]
Heated	945.02 ± 25.45[b]	1059.22 ± 21.57[a]	1000.15 ± 40.11[ab]	982.33 ± 36.52[ab]
BWG, g				
Unheated	1128.83 ± 19.32[a]	1104.50 ± 9.39[a]	1078.67 ± 25.76[a]	1097.00 ± 21.15[a]
Heated	170.19 ± 2.29[a]	149.25 ± 3.78[b]	132.04 ± 4.18[c]	146.04 ± 8.90[bc]
FE, %				
Unheated	0.73 ± 0.12[b]	0.72 ± 0.19[b]	0.70 ± 0.18[b]	0.83 ± 0.16[a]
Heated	0.54 ± 0.21	0.58 ± 0.21	0.57 ± 0.25	0.57 ± 0.15

Abbreviation: FI – feed intake, BWG – body weight gain, FE – Feed efficiency, Control − basal diet with untreated drinking water, ERW − basal diet and electrolyzed reduced drinking water, TOCO − tocotrienol diet (2% scum rice bran oil + 3% soybean oil) with untreated drinking water, VITE − basal diet + vitamin E at 50 mg/kg with untreated drinking water.Results are from 6 birds per treatment. Values with different letters are significantly different ($p < 0.05$). Duncan's least significance multiple-range test was applied to compare means.

The oxidative stability of pectoralis muscle is presented in Table 2. MDA production was significantly ($P < 0.05$) decreased in unheated chickens fed VITE and TOCO when compared to control and ERW. Diet had significant effect on liver and plasma MDA levels among unheated and heated birds. The unheated TOCO chicks showed significantly lower liver MDA concentration than other groups. The heat challenge resulted in a significantly higher liver MDA levels in the control chicks when compared to other groups. Heat-treated birds did not show any changes in plasma MDA levels with dietary modulation. However, prior to heat treatment, plasma MDA levels were significantly decreased by TOCO and VITE supplemented diets compared to the control diet group.

Table 2. MDA concentrations in skeletal muscle, liver and plasma of broiler chickens fed the experimental diets.

MDA concentration	Control	ERW	TOCO	VITE[1]
nmol/g muscle				
Unheated	68.92 ± 3.67[a]	62.60 ± 4.38[a]	27.62 ± 1.80[b]	24.09 ± 2.69[b]
Heated	78.94 ± 2.32[a]	46.66 ± 3.39[b]	30.07 ± 3.01[c]	39.30 ± 2.56[b]
nmol/g liver				
Unheated	154.33 ± 3.67[a]	152.02 ± 5.43[a]	131.03 ± 4.72[d]	137.60 ± 14.75[b]
Heated	170.19 ± 2.29[a]	149.25 ± 3.78[b]	132.04 ± 4.18[c]	146.04 ± 8.90[bc]
nmol/ml plasma				
Unheated	25.38 ± 0.78[a]	20.34 ± 1.52[ab]	12.55 ± 1.59[c]	16.88 ± 1.90[bc]
Heated	21.42 ± 3.53[ab]	20.48 ± 1.52[ab]	18.03 ± 1.70[bc]	15.79 ± 1.21[bc]

Abbreviation: MDA – malondialdehye, Control − basal diet with untreated drinking water, ERW − basal diet and electrolyzed reduced drinking water, TOCO − tocotrienol diet (2% scum rice bran oil + 3% soybean oil) with untreated drinking water, VITE − basal diet + vitamin E at 50 mg/kg with untreated drinking water.Results are from 6 birds per treatment. Values with different letters are significantly different ($p < 0.05$). Duncan's least significance multiple-range test was applied to compare means.

Discussion

High ambient temperature, whether 'acute' or 'chronic' in nature, significantly hinders growth of animals. Reduced growth has been considered a problem of reduced feed intake (Hurwitz et al., 1980; Howlider and Rose, 1987). Feed intake is inversely related to environmental temperature in chickens (McDonald et al., 1981; Suk and Washburn, 1995). It is believed that for every 10°C increase in ambient temperature above 20°C, there is a 17% reduction in feed intake (Austic, 1985). Furthermore, reduced efficiency is a common phenomenon in hot conditions. Azad et al. (2010c) observed a 66% reduction in body weight and a 50% reduction in feed intake when birds were exposed to 34°C from 2 to 4 wk of age, and suggested that broiler body weight gain is reduced due to reductions in both feed consumption and the efficiency of feed utilization under constant high temperature. The present results indicated that body weight gain and feed intake were significantly reduced in untreated heat-stressed broilers compared to untreated control broilers maintained at 24ºC. These results were in agreement with previous reports (Donkh, 1989; Lu et al., 2007; Azad et al., 2010a) who reported that the reduced performance of broiler chickens is manifested in the form of decreased weight gain and feed consumption under chronic heat treatments.

The present results suggest that body weight gain and feed intake could be significantly improved by providing electrolyzed reduced water to chickens subjected to heat stress for two weeks. Our previous studies also showed a dramatic response of electrolyzed reduced water with respect to weight gain and feed consumption of broiler chickens upon exposure to a 5 d period chronic heat stress conditions (unpublished data). In situations where feed consumption is increased, activity of feed forward enzyme like CS (citrate synthase) may be elevated, which may lead to TCA cycle modulation for the increase of energy production. Indeed, heat-stressed broilers supplied with electrolyzed reduced water exhibited higher CS activity (unpublished data), indicating that the reduced water could improve energy production via modulation of TCA cycle. In the current study, we found that with dietary supplementation of tocotrienol had negligible influence on body weight gain and feed intake. These finding are not in agreement with earlier work which demonstrated diet supplemented with tocotrienol produced either a decrease (Qureshi et al., 2011) or an increase in chickens weight gain (Yu et al., 2006). Body weight gain and feed intake were not significantly influenced by dietary vitamin E supplementation under heat stress condition. Similar results have also been reported by Niu et al. (2009). Prior to heat treatment, the feed efficiency of vitamin E chicks was significantly higher than that of other diets. This result was consistent with the result reported by Guo et al. (2003).

It was reported that either constant (Azad et al., 2010a) or cyclic (Feng et al., 2008) heat exposure may result 25-30% increments in breast muscle MDA levels; suggesting oxidative damage to skeletal muscle is variable under chronic heat exposure. In the present study, the MDA content in the skeletal muscle of heat-exposed untreated broilers increased by 15% as compared to the untreated controls. It is well documented that the characteristics of electrolyzed-reduced water as a potent antioxidant which could effectively scavenge hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot-}$) and hydroxyal radical ($^{\cdot}OH$), and protect DNA, RNA, proteins, cells, and tissues under strong oxidative stress conditions (Shirahata et al., 1997; Lee et al., 2006; Tsai et al., 2009; Park et al., 2009). The results of the present study also confirmed that the reduced water had the ability to lower oxidative damage by scavenging H_2O_2 and $^{\cdot}OH$ radicals from skeletal muscle in chickens exposed to chronic heat stress. Our previous studies also showed that electrolyzed reduced water could inhibit ROS-induced oxidative damage to skeletal muscle of broiler chickens upon exposure to a 5 d period chronic heat stress conditions (unpublished data). Recent studies have shown that tocotrienol had better anti-oxidative, anti-hypercholesterolemic, and anti-cancer activities than those of tocopherol (Sen et al., 2007). Broilers fed a diet supplemented with tocotrienol had significantly reduced MDA levels in the breast muscle, liver and plasma irrespective of thermal load. This findings concur those of Lanari et al. (2004) and Kang et al. (2001). The supplementation of basal diet with vitamin E at 50 mg/kg for 7 weeks in broilers has been shown to a decrease in lipid peroxidation in tissues (Bartov and Frigg, 1992). Sahin et al. (2001) found that birds received basal diet supplemented with vitamin significantly inhibited MDA concentrations in both liver and plasma of broiler chickens reared under 32°C thermal conditions. Moreover, diets supplemented with vitamin E significantly reduced lipid peroxidation in breast muscle than non-supplemented vitamin E chicks (Avanzo et al., 2001). Hence it appears that vitamin E supplementation could reduce MDA content in various organs of broilers reared under constant high temperature condition.

In conclusion, our results suggested that antioxidants used in this experiment are capable to scavenge free radicals to lower oxidative damage in whole body of the broilers. However, they could not effectively improve growth performance except electrolyzed reduced water treatment under chronic heat stress conditions. Therefore, electrolyzed reduced water can be considered as a protective management practice for reducing the negative impact of heat stress.

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