Ameliorative effects of betaine and ascorbic acid on erythrocyte osmotic fragility and malondialdehyde concentrations in broiler chickens during the hot-dry season

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ABSTRACT

The study evaluated the effect of betaine and ascorbic acid (AA) administration on the erythrocyte osmotic fragility (EOF) and malondialdehyde (MDA) concentration of broiler chickens during the hot-dry season. Eighty-day-old broilers were divided into four groups: Group I (control) given sterile water; Group II, betaine (250 mg/kg); Group III, AA (50 mg/kg); and Group IV, betaine (250 mg/kg) + AA (50 mg/kg) orally for 42 days. Blood samples were collected from each bird with and without anticoagulant, sodium ethylenediaminetetraacetate, on days 21 and 42, for the determination of EOF and serum MDA concentrations. The dry-bulb temperature (DBT), relative humidity and temperature-humidity index (THI) recorded were 28.3–35.67°C, 69.00–93.00% and 28.18–34.82, respectively. The results showed that betaine + AA (7.78 ± 9.35%) significantly (P < .05) reduced EOF, compared to control birds (16.27 ± 9.35%) at 0.7% on day 21. MDA concentrations of broiler chickens in the betaine (1.37 ± 0.038 nmol/L), AA (1.41 ± 0.039 nmol/L) and betaine + AA (1.41 ± 0.040 nmol/L) groups during the experimental period were significantly (P < .05) lower when compared with that of the control group (1.54 ± 0.043 nmol/L). It is concluded that the co-administration of betaine and AA to broiler chickens decreased EOF and MDA during the hot-dry season.

1. Introduction

Broiler chicken production under high ambient temperature (AT) and high relative humidity (RH) has been shown to induce heat stress (Ali et al. 2010). Heat stress results in the excess production of reactive oxygen species (ROS) and, consequently, oxidative damage (Zhang et al. 2014). Under such adverse conditions, the body is not able to synthesize the enzymes required to destroy ROS or repair the damage. The hot-dry season, characterized by high AT and high RH, in the Northern Guinea Savannah zone has been shown to be thermally stressful to poultry (Sinkalu et al. 2015).

Erythrocyte osmotic fragility (EOF) is influenced by temperature (Oyewale et al. 2011). It has been demonstrated to be a biomarker of oxidative stress (Adenkola & Ayo 2009), and a diagnostic tool for evaluating transport-induced oxidative stress in quail (Minka & Ayo 2013) and rabbits (Ayo et al. 2015). Erythrocyte membrane could be damaged by lipid peroxidation, resulting from increased generation of ROS (Azeez et al. 2012), and measured by the malondialdehyde (MDA) concentration (Roberts & Sindhu 2009). Increased MDA concentration is involved in the pathogenesis of certain conditions in broiler chickens such as aflatoxicosis (Chen et al. 2013), structural injury and decreased immune function of the spleen due to low selenium intake (Peng et al. 2012).

Betaine is a powerful osmotic stress protectant (Bremer 2014) and a metabolite of choline (Lever & Slow 2010; Mitsuya et al. 2013). It is synthesized from choline by choline oxidase in the liver (Igwe et al. 2015). It plays a vital role in the integrity of cell membranes, methylation reactions and memory development (Hogeveen et al. 2013). Ascorbic acid (AA, vitamin C) is primarily synthesized in the chicken by the kidneys, but during heat stress, endogenous AA becomes insufficient to meet the bird’s requirements (Abidin & Khatoon 2013). AA supplementation to poultry, including broiler chickens, improves feed intake, feed efficiency, weight gain, nutrient digestibility, immune response and antioxidant status (Khan et al. 2012). However, Jena et al. (2013) demonstrated that combined administration of two antioxidants, namely, vitamins C and E, enhanced erythrocytic antioxidant status more than their individual administrations in coloured broiler breeder hens under the stressful hot-humid conditions. Although studies have been conducted on the single effects of betaine and, especially, AA in broiler production, there is a paucity of information on their combined effects following heat stress on broiler chicken.

The aim of the study was to evaluate the ameliorative effects of the co-administration of betaine and AA on EOF and MDA concentration in broiler chickens during the hot-dry season.

2. Materials and methods

2.1. Experimental site and meteorological conditions

The experiment was performed at a poultry house in Samaru, Zaria (11°10′N, 07°38′E), located in the Northern Guinea Savannah zone of Nigeria. It was carried out from April to May 2013,
2.2. Experimental animals, grouping and management

Eighty birds, purchased at day-old from a commercial farm, were used for the experiment. The broiler chickens were fed with commercial feed (broiler pre-starter (days 0–14), broiler starter (days 15–28) and broiler finisher (days 29–42)) (Table 1). They were vaccinated against infectious bursal disease (at days 7 and 14) using Gumboro vaccine, and against Newcastle disease (at day 21) using Lasota vaccine. All vaccines were administered via drinking water. The birds were housed in the same pen, littered with wood shavings on a concrete floor and having a zinc roof with cardboard ceiling. The dimension of the pen was 8.4 m × 5.6 m × 1.91 m and the birds were stocked at 0.35 m² per bird. The pen was partitioned using plywood into four cubicles to house each group. It was provided with biosecurity measures, including a footbath and non-accessibility to non-essential persons, animals, birds and rodents. Farm wears security measures, including a footbath and non-accessibility to non-essential persons, animals, birds and rodents. Farm wears security measures, including a footbath and non-accessibility to non-essential persons, animals, birds and rodents. Farm wears security measures, including a footbath and non-accessibility to non-essential persons, animals, birds and rodents. Farm wears security measures, including a footbath and non-accessibility to non-essential persons, animals, birds and rodents.

2.2.3.1. Experimental drugs and their preparation

The AA (Sigma Chemical, St. Louis, MO, USA) was dissolved in 5 mL of distilled water and administered at the dose of 250 mg/kg (Pillai et al. 2006), Group II was administered with betaine hydrochloride only at 50 mg/kg (Sinkalu et al. 2008), while Group IV (betaine + AA) was co-administered with betaine and AA at 250 mg/kg and 50 mg/kg, while the content of betaine hydrochloride capsules (Twinlab, Isi Brands Incorporated, American Fork, UT, USA), after carefully removing the capsules, was dissolved in 5 mL of distilled water and administered at 250 mg/kg. Betaine and AA were administered during the morning period at 06:00 h and 07:00 h, to avoid excessive stress on the birds. Four trained persons administered the agents to each group, and it took 15 s to administer each of the agents to the birds in each experimental group.

2.3. Experimental measurements

2.3.1. Evaluation of serum MDA concentration

Serum MDA concentration was evaluated as an index of lipid peroxidation as described by Janero (1990). Briefly, serum sample (100 μL) was mixed with sodium dodecyl sulphate-acetate buffer (pH 3.5) and an aqueous solution of thiobarbituric acid. After heating at 95°C for 60 min, the red pigment produced was extracted with an n-butanol–pyridine mixture and estimated by measuring the absorbance at 532 nm. Tetramethoxy-propane was used as an external standard and MDA concentration was expressed in nmol/L.
2.4. Statistical analysis

The data obtained were expressed as mean ± standard error of the mean, using Graphpad 4.0 for windows (San Diego, CA, USA). The values obtained were subjected to statistical analysis using repeated-measures analysis of variance, followed by Tukey’s post hoc test. Values of \( P < .05 \) were considered significant (Snedecor & Cochran 1994).

3. Results and discussion

3.1. Determination of EOF

On day 21 and at 0.7% NaCl concentration, the decrease in EOF in all the treated groups (9.35 ± 2.71%; 6.98 ± 2.47% and 7.78 ± 1.66% for betaine, AA and betaine + AA groups, respectively), when compared respectively with the control group (16.27 ± 9.35%), was significant \( (P < .05) \). There were no significant decreases in percentage EOF at 0.5% NaCl concentration in the experimental groups, when compared with that of the control (Figure 1). On day 42, at 0.7% NaCl concentration the percentage of EOF (3.97 ± 1.05%) decreased significantly \( (P < .05) \) in the betaine group, relative to the value in the control group (11.04 ± 2.09%). The EOF values of 6.23 ± 2.01% and 7.36 ± 0.97% obtained in betaine + AA and AA-treated groups, respectively, did not differ, when compared to the value in the control group (11.04 ± 2.09%). At 0.5% NaCl concentration, the EOF value (15.14 ± 4.38%) recorded in the betaine group decreased significantly \( (P < .01) \) when compared to the value (33.58 ± 4.48%) obtained in the control group (Figure 2).

3.2. Variations in MDA concentration

The MDA concentration in broiler chickens administered with AA (1.31 ± 0.04 nmol/L) was significantly \( (P < .05) \) lower than that recorded in the control group (1.50 ± 0.066 nmol/L). However, the decreases in MDA concentrations in the betaine (1.43 ± 0.036 nmol/L) or betaine + AA (1.40 ± 0.062 nmol/L) groups were not significant \( (P > .05) \) on day 21 of the experimental period (Figure 3). On day 42, the MDA concentration was lower \( (P < .05) \) in the betaine (1.31 ± 0.068 nmol/L) group than in the control (1.59 ± 0.055 nmol/L) and in any of the treatment groups. The MDA concentration in betaine + AA group (1.41 ± 0.053 nmol/L) was significantly \( (P < .05) \) lower than that of the control (1.59 ± 0.055 nmol/L) or AA-treated group (1.51 ± 0.040 nmol/L) (Figure 3). Overall, the MDA concentration of broiler chickens in any of the treatment groups during the experimental period was significantly \( (P < .05) \) lower when compared with that of the control group (Figure 4).

![Figure 1. Variations in EOF of broiler chickens on day 21 of the study period (n = 20) \(^{a,b,c}\) Means with different superscript letters are significantly \( (P < .05) \) different; Group I = control; Group II = betaine administration; Group III = AA administration; Group IV = co-administration with betaine and AA.](image1)

![Figure 2. Variation in EOF of broiler chickens on day 42 during the study period (n = 20) \(^{a,b,c}\) Means with different superscript letters are significantly \( (P < .05) \) different; Group I = control; Group II = betaine administration; Group III = AA administration; Group IV = co-administration with betaine and AA.](image2)
4. Discussion

From the results of the present study, it can be observed that the DBT values obtained were outside the thermoneutral zone for broiler chickens above four weeks old, which is 18–21°C (Aengwanich & Simaraks 2004). The RH values obtained were predominantly outside the optimal range of 65–70% for broilers between the ages of three and seven weeks (Akyuz & Boyaci 2010). The findings of the present study agree with that of de Oliveira et al. (2013), who showed that the optimum AT and RH values for broiler chickens are 21°C and 74%, respectively. The high THI recorded in the present study, with values exceeding 20.8 (Purswell et al. 2012), showed that the thermal environmental conditions were stressful to the broiler chickens. This thermally stressful condition is classical of the hot-dry season in the zone, and increases the risk of adverse effects of heat stress on the well-being of the birds (Sinkalu et al. 2008). This agrees with the finding of Wahab et al. (2010), who showed that heat stress increases the risk of haemolysis in rats during the hot-dry season. The results of the present study show that heat stress induced significant haemolysis in broiler chickens. The findings of the present study also agree with those of Sinkalu et al. (2015), who showed that high thermal environmental conditions in poultry housing adversely affect the EOF of broiler chickens during the hot-dry season in the zone. THI is an index of thermal comfort. It integrates the effects of temperature and humidity and may be used as a tool to predict the effects of thermal conditions on the performance of broiler chickens (Purswell et al. 2012).

The damage of the erythrocyte membrane may be due to the established finding that heat stress increases ROS generation (Ramnath et al. 2008), resulting in lipid peroxidation. Haemolysis may also be due to the denaturing of plasma membrane proteins during heat stress (Jóźwiak & Leyko 1992). Furthermore, in birds, including broiler chickens, heat stress induces loss of carbon dioxide through respiration, bicarbonate ions due to panting and monovalent cations (especially sodium and potassium) through urine, altering the acid–base balance (Ahmad & Sarwar 2006). The imbalance in pH of the erythrocyte environment adversely affects EOF (Oyewale et al. 2011). Erythrocytes of heat-stressed broiler chickens are longer and thinner than those not subjected to heat stress (Maxwell et al. 1992). These may necessitate the administration of antioxidants such as betaine and/or AA to broiler chickens under heat stress conditions.

The results of the present study demonstrate that the co-administration of betaine and AA exerted antioxidant effects on the erythrocyte membrane of heat-stressed broiler chickens during the hot-dry season in the zone. Betaine has been described to show antioxidant potentials on the carcass of broiler chickens reared under heat stress conditions (Alirezaei et al. 2012). It has been demonstrated to have a dipolar
zwitterion potential, resulting in its osmoprotective properties at the cellular potential. It is also involved in transmethylation reactions in organisms. Betaine as a methyl donor provides its labile methyl groups for the synthesis of several metabolically active substances such as creatine and carnitine (Eklund et al. 2005). The physiological and biochemical potentials of AA are due to its action as an electron donor. The ability to donate one or two electrons makes it an excellent reducing agent and antioxidant (Du et al. 2012). AA has also been demonstrated to exert antioxidant effects in pullets (Minka & Ayo 2010) and ostrich chicks (Minka & Ayo 2012) transported under thermally stressful hot-dry season. However, the effects of the co-administration of betaine and AA, which was the emphasis of the present study, indicate that both agents (betaine and AA) exerted their antioxidant effect on day 21 of the experimental period. This was evidenced by a significantly lower EOF observed in the broiler chickens administered with betaine + AA, as well as a decrease in the MDA value obtained in the same combination group. This finding is in agreement with those of Bai et al. (2014), who showed that the combination of antioxidants had better results than their individual administrations.

Furthermore, the antioxidant effect of the combination of betaine and AA was apparent in the broiler chickens on day 42 of the experimental period, evidenced by a lower EOF at 0.7% and decreased MDA concentration in the birds treated with betaine + AA. This further demonstrates that the combined administration of betaine and AA to heat-stressed broiler chickens may confer some protection on the birds against the adverse effects of the thermally stressful hot-dry season in the zone. This finding is in agreement with the results obtained by Azeez et al. (2011), who demonstrated that AA and/or vitamin E decreased the adverse effects of oxidative stress on the erythrocyte membrane of domestic chickens. The ability of both betaine and AA to reduce the adverse effects of heat stress-induced oxidative damage on the erythrocyte membrane may be due to their ability to scavenge ROS, generated in excess during thermally stressful conditions in the Northern Guinea Savannah zone. The antioxidant effect of both agents (betaine + AA) is beneficial in reducing haemolysis, thereby increasing the oxygen-carrying capacity of the erythrocytes in broiler chickens, reared under heat stress conditions.

5. Conclusion

In conclusion, the co-administration of betaine and AA decreased EOF and MDA in broiler chickens reared during the hot-dry season.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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