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Research

Effects of betaine and ascorbic acid on tonic immobility, superoxide dismutase and glutathione peroxidase in broiler chickens during the hot-dry season

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ABSTRACT

The aim of the study was to evaluate effects of betaine and ascorbic acid (AA) on tonic immobility (TI), and activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in broiler chickens, reared during the hot-dry season. Eighty White Ross breed day-old broilers were divided into 4 groups. Group I (control) was given sterile water; group II, betaine (250 mg/kg); group III, AA (50 mg/kg), and group IV, betaine + AA, at 250 + 50 mg/kg, respectively. The administration was done orally in drinking water and daily for 42 days. The duration of TI of each bird was measured. Serum obtained from blood samples collected from the birds were assayed for activities of the enzymes. The dry-bulb temperature (28.33°C-35.67°C), relative humidity (69.0-93.0%), and temperature-humidity index (27.85-36.1), recorded during the study period showed that the birds were subjected to heat stress. Betaine and its coadministration with AA decreased ($P < 0.05$) TI duration compared to the control group. Betaine + AA significantly increased ($P < 0.01$) SOD activity compared with the controls. There were significant increases in GPx activity in broiler chickens in betaine ($P < 0.01$), AA ($P < 0.05$), and betaine + AA ($P < 0.001$) groups, when compared with that of controls. The result demonstrates that betaine and/or AA decreased TI and enhanced activities of the antioxidant enzymes in heat-stressed birds. It is concluded that the administration of betaine and/or AA to broiler chickens alleviates the adverse effects of heat stress by decreasing fearfulness and increasing activities of SOD and GPx enzymes.

Introduction

High ambient temperature and high relative humidity (RH) occurring during the hot-dry season have been shown to cause heat stress in birds (Ayo et al., 2011; Minka and Ayo, 2012). Heat stress is a major environmental stressor, posing a serious challenge to the success of poultry industry in the hot-humid regions of the world. The understanding of the biochemical changes in birds may be of value in controlling the negative effects caused by heat stress it causes (Lara and Rostagno, 2013). One component of stress in poultry is fear, and the duration of tonic immobility (TI) is the physiological indicator of fear. TI is less affected by external influences under the action of compulsory fixation. TI duration is also related to aggressive behavior, pecking, and cannibalism among hens in commercial poultry industry; hence, it is considered as an indirect diagnostic tool to assess stress, such as heat stress, in poultry (Tikhonov and Miftakhutdinov, 2014). TI is also an adaptive psychophysiological response (Sanotra et al., 2002). Studies have shown that genetic predispositions of poultry influence responses of birds to heat stress in terms of behavior and production (Mack et al., 2013). Birds confronted with treats exhibit fear-induced freezing called TI, mostly observed in prey species as a defense mechanism (Abe et al., 2013). Forkman et al. (2007) defined fear as an adaptive behavior which protects the animal from psychochemical damage, eliciting a reaction to the perception of actual

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Table 1
Proximate analysis of the broiler diet during the study period

Parameters	Prestarter	Starter	Finisher
Percentage dry matter	93.84	93.73	94.14
Metabolizable energy	3,144.34	3,406.07	3,357.16
Percentage crude protein	18.12	16.62	13.75
Percentage crude fiber	5.6	3.36	4.38
Percentage oil	5.45	5.08	5.26
Percentage ash	11.96	6.95	7.4
Percentage nitrogen-free extract	58.07	67.99	69.21

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danger. It is important to understand the behavior of animals because it affects their production and welfare (Broom, 2011). It is practically impossible to completely eliminate fear-inducing (stressful) situations during the rearing of birds (Lábaque et al., 2013), adversely affecting their welfare and production (de Haas et al., 2013). Zulkifli et al. (2009) described broiler chickens showing short or long TI responses as low-fear or high-fear responders, respectively. Antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GPx), terminate free-radical reactions in cells by using a chain-reaction mechanism (Benzie, 2003). Enzymatic antioxidants like SOD and GPx scavenge both intracellular and extracellular oxidants, and prevent lipid peroxidation of cytomembranes (Sunil Kumar et al., 2011).

Betaine is widely found in nature, including animal tissues, seafood (from marine invertebrates), sugar beets, wheat bran, spinach, and microorganisms. It protects cells, protein, and enzymes from deleterious effects of environmental stress factors (like high ambient temperature, low water, high salinity) because it is an osmolyte (Craig, 2004). Betaine has been shown to enhance antioxidant defense, decreasing lipid peroxidation in the breast muscles of broiler chickens (Alirezaei et al., 2012). Folate metabolism is adversely affected by insufficient serum levels of methyl donor such as betaine, resulting in decline in methylation and antioxidant capacity (Hamlin et al., 2013). It has been demonstrated that AA supplementation to heat-acclimated broiler chickens ameliorated the adverse effect of heat stress (El-Habbak et al., 2011). Ascorbic acid (AA) decreases the lipid oxidation in meat of broiler chickens stored for some days (Skřivan et al., 2012). It improves the breast meat yield in broilers during the hot-dry season (Abioja et al., 2011) and reduced the incidence of hypoxemia and susceptibility of broilers to heart failure (Nain et al., 2008). Glutathione plays a crucial role in controlling AA metabolism by regulating the expression and subcellular localization of the transporters, involved in the uptake of AA from extracellular sources (Mardones et al., 2012). Coadministration of antioxidants may be more effective than administration of single antioxidant because the combined administration has been shown to act synergistically to produce a magnified effect (Bai et al., 2014).

The aim of the study was to evaluate effects of coadministration of betaine and AA on TI, and the activities of SOD and GPx in broiler chickens during the hot-dry season.

Materials and methods

Experimental site and thermal environment conditions

The experiment was carried out in a poultry house in Samaru—Zaria (11°10′N, 07°38′E), located in the Northern Guinea Savannah zone of Nigeria during the hot-dry season, from April to May, 2013 (Dzenda et al., 2013).

Experimental birds and management

Eighty male broiler chickens, belonging to the White Ross breed, were purchased at day old from a commercial hatchery in Iperu-Remo (6°56′N, 3°43′E), Nigeria. Tags were tied to their legs for identification. The broiler chickens were maintained on commercial feed as follows: broiler prestarter (day 0-14), broiler starter (day 15-28), and broiler finisher (day 29-42). The proximate analysis of the feeds given is shown in Table 1, and water consumption by the birds is shown in Table 2. The birds were vaccinated, via drinking water, against infectious bursal disease (on days 7 and 14), using Gumboro vaccine; and Newcastle disease (on day 21), using Lasota vaccine. They were housed in a poultry pen, littered with wood shavings on concrete floor and measuring 8.4 × 5.6 × 1.91 m. The system of management complied with new European Union council directive 99/74/EC to phase out the conventional bird cage by year 2012. The stocking density was reduced to 4 birds/m^2, lower than 10-15 birds/m^2 described by Muniz et al. (2006) to eliminate the effects of stocking density on the results of the experiment. The pen, having a zinc roof with cardboard ceiling, was partitioned into 4 cubicles to house each group of birds, divided into 4 groups (groups I-IV). Group I served as the control group and was given only sterile water. Group II was administered with betaine hydrochloride (Twinlab, Isi Brands Incorporated, American Fork, UT) only at 250 mg/kg (Pillai et al., 2006), group III was given AA (Sigma Chemical, St. Louis, MO, U.S.A.) only at 50 mg/kg (Sinkalu et al., 2008), whereas group IV was coadministered with betaine hydrochloride (250 mg/kg) and AA (50 mg/kg). Betaine hydrochloride and AA were administered to each bird daily by oral route for 42 days. Betaine hydrochloride (650 mg) was dissolved at a concentration of 32,500 mg/L of water an administered at dose of 250 mg/kg (Pillai et al., 2006), whereas AA (100 mg) was dissolved at a concentration of 5000 mg/L of water and was administered at the dose of 50 mg/kg (Sinkalu et al., 2008).

Tonic immobility

The different durations of TI, which is evidence that birds respond differently and behaviorally to stress, were assessed (Wang et al., 2013). This was performed by catching gently each bird and

Table 2
Water consumption (L) of broiler chickens administered with betaine and/or ascorbic acid during the hot-dry season

Week	Group I	Group II	Group III	Group IV
1	0.91 ± 0.01 (0.43-1.17)	0.80 ± 0.11 (0.43-1.33)	0.84 ± 0.10 (0.36-1.28)	0.78 ± 0.13 (0.33-1.26)
2	1.30 ± 0.15 (0.57-1.81)	1.24 ± 0.17 (0.39-1.78)	1.35 ± 0.17 (0.54-1.88)	1.26 ± 0.16 (0.53-1.71)
3	2.11 ± 0.26 (1.31-3.06)	2.03 ± 0.24 (1.31-3.03)	2.03 ± 0.26 (1.00-3.13)	1.95 ± 0.25 (0.92-2.83)
4	3.51 ± 0.50 (1.25-5.42)	3.29 ± 0.49 (1.33-5.15)	3.44 ± 0.45 (1.58-5.42)	3.51 ± 0.45 (1.58-5.42)
5	5.02 ± 0.40 (3.52-6.18)	4.75 ± 0.39 (3.89-6.42)	4.81 ± 0.40 (3.63-6.00)	4.56 ± 0.43 (3.46-6.33)
6	6.87 ± 0.33 (5.02-7.68)	6.44 ± 0.40 (4.65-7.98)	7.09 ± 0.29 (5.67-8.17)	6.68 ± 0.33 (4.83-7.42)

Values in parenthesis are minimum—maximum, n = 20. Group I = control; group II = betaine administration; group III = ascorbic acid administration; group IV = coadministration with betaine and ascorbic acid.