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Research Article

Ascorbic Acid Supplementation Effect on Haematology and Oxidative Stress Parameters of Broiler Chicken during the Hot-Dry Season in Southern Guinea Savannah

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ABSTRACT: This study was done to investigate the effect of inclusion of ascorbic acid (AA) in drinking water of broiler chicken on haematological and oxidative stress parameters during the hot-dry season. Sixty day old chicks were used for the experiment, and were assigned to two dietary treatments with 30 birds per treatment and five replicate in a completely randomized design. Treatment 1 was the control with no supplements, treatment 11 was supplemented with AA at the rate of 500 mg/L in drinking water termed the experimental group. On the last day (58^{th}) of the experiment half of the birds were randomly selected from each replica group and blood sample (2 ml) was obtained through the wing web and divided into 2 parts; one for determination of haematological parameters while the second part was centrifuged and the resultant serum harvested and stored. The results indicated that the values of PCV (24.25 ± 2.80%), haemoglobin (8.08 ± 0.83 gm/dl) and total erythrocyte count (4.35 ± 1.13 x 10^6/ l) obtained in control were all significantly lower than the recorded corresponding value in the experimental group 31.50 ± 13.13%; 10.50 ± 4.38 gm/dl and 5.94 ± 1.28 x 10^6/ l) respectively. The recorded values of malondialdehyde obtained in control (2.18 ± 0.22 ng/ml) was significantly higher than the values recorded in experimental group (0.93 ± 0.09) while the values of superoxide dismutase, catalase and glutathione peroxidase were significantly higher in the control than the obtained values experimental group. The fragiligram shifted more to the left in the control than the experimental group and was statistically significant. In conclusion AA administration mitigate the adverse effects brought about by heat stress in broiler chicken; its supplementation is thus recommended during the hot-dry season to alleviate heat stress.

Keywords: Ascorbic acid, heamatology, oxidative stress parameters, broiler chicken, hot-dry season

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Askorbik Asit İlavesinin Güney Gine Savana'da Sıcak-Kurak Mevsimde Broylerlerde Hematolojik ve Oksidatif Stres Parametreleri Üzerine Etkileri

ÖZ: Bu çalışmada sıcak-kuru mevsimde broyler civcivlerin içme sularına askorbik asit (AA) ilavesinin hematolojik ve oksidatif stres parametreleri üzerine etkisi araştırılmıştır. Denemede 60 adet, bir günlük yaşta civcivler kullanılmış ve 5 tekerrürlü, 30 civciv içeren 2 deneme grubuna tesadüf deneme düzeninde yerleştirilmişlerdir. Kontrol grubu olan birinci gruba herhangi bir ilave yapılmamıştır, ikinci deneme grubunun içme sularına 500 mg/lt düzeyinde AA ilave edilmiştir. Denemenin son gününde (58. gün) her alt gruptaki piliçlerin yarısı seçilmiş ve kanat altı venlerinden kan numuneleri alınmıştır. Alınan kanların yarısı hematolojik parametreler için ayrılmış, diğer yarısı da santrifüj edilerek serumları ayrılıp saklanmıştır. Sonuçlar, kontrol grubunda PCV (%24.25 ± 2.80), hemoglobin (8.08 ± 0.83 gm/dl) ve toplam eritrosit sayısı (4.35 ± 1.13 x 10^6/ l) değerlerinin sırasıyla %31.50 ± 13.13; 10.50 ± 4.38 gm/dl ve 5.94 ± 1.28 x 10^6/l olan deneme grubundan önemli derecede daha düşük olduğunu göstermiştir. Kontrol grubundan elde edilen malondialdehit düzeyi (2.18 ± 0.22 ng/ml) deneme grubunda kaydedilen değerden (0.93 ± 0.09 ng/ml) önemli derecede daha yüksek iken süperoksit dismutaz, katalaz ve glutasyon peroksidaz değerleri kontrol grubunda deneme grubuna göre önemli derecede daha yüksek bulunmuştur. Sonuç olarak AA uygulaması broyler piliçlerde sıcak stresinin olumsuz etkilerini hafifletmiştir. Bu nedenle sıcak-kuru mevsimlerde sıcak stresinin etkilerini hafifletmek için AA ilavesi önerilmektedir.

Anahtar Kelimeler: Askorbik asit, hematolojik, oksidatif stress parametreleri, broyler, sıcak-kuru mevsim

INTRODUCTION

Homeothermic animals (depending on their physiological state) have a thermoneutral zone where energy expenditure to maintain normal body temperature is minimal, constant and independent of environmental temperature (1). Global warming as a result of increased industrialization and environmental degradation has led to continuous increase in ambient temperature thereby making heat stress a major problem of livestock farming particularly in the poultry sector (2). When environmental variables, such as ambient temperature, humidity, air movement and solar radiation combine with, reach value that surpass the upper limit of the thermoneutral zone, animals enter a condition known as heat stress (3). In the tropical environment, meteorological factors exert significant influence on domestic birds. Direct meteorological factors affecting birds include high ambient temperature and high relative humidity resulting in severe heat stress (4) which may alters many physiological parameters in livestock (5), hence, resulting in alteration in body homeostasis. Heat stress occurs when the core body temperature of a given species exceeds its range specified for normal activity resulting from a total heat load (internal production and environment) exceeding the capacity for heat dissipation. Under this stressful

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environmental condition, the concentration of antioxidant vitamins decreases in the plasma and tissues leading to damage of cell membrane (6) as well as generation of free radicals which may impair homeostasis mechanisms resulting into pathological changes (7) and also play a pivotal role in tissue damages as well as adverse effects on erythrocyte (8, 9). This ravaging action of free radical can be quenched by antioxidants (10, 11).

Antioxidants terminate the chain reaction by remaining free radical intermediates, and inhibit other oxidative reaction, and they do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols (12). Normally, non-enzymatic antioxidants, such as vitamin C, produced in the bird's kidneys, are involved in the elimination of excess free radicals from the body, but under praxis condition, they are either exhausted or overwhelmed; thus, exposing cells to their harmful effects (13). Several researchers have suggested various ways of ameliorating the deleterious effects of heat stress acting on birds and nutritional manipulations through the use of electrolytes (minerals, oils and vitamins) (14). Ascorbic Acid (AA) Supplementation Has Been Shown To Be Beneficial against heat stress (15, 16) and stress-induced tissue damages (17).

However, the aim of this study is to investigate the effect of administration of AA as an antioxidant on haematology and some biomarker of oxidative stress parameters in broiler chicken during the hot-dry season.

MATERIALS AND METHODS

The experiment was carried out at the Poultry Unit of Teaching and Research farm of University of Agriculture Makurdi (07°41/ N, 08° 37/ E) in the Southern Guinea Savannah zone of Nigeria. The work was done during the hot-dry season (February - April).

Experimental Design

Anak 2000 broiler day old chicks numbering 60 were used for the experiment, and these were purchased from a reputable hatchery. The birds were assigned to three dietary treatments with 20 birds per treatment and four replicate in a completely randomized design. Treatment 1 was the control with no supplements, while treatment 2 was supplemented with ascorbic acid (AA) at the rate of 500 mg /L in drinking water (15). The chicks were fed and provided with water *ad libitum*. The experiment lasted eight weeks and during this period they were subjected to standard management procedure with vaccination against Common diseases. On the 58th day of the experiment half of the birds were randomly selected from each dietary group and blood sample (2 ml) was obtained aseptically through the wing web and divided into 2 parts, that which is meant for determination of haematological parameters was immediately poured inside a sample bottle, containing an anticoagulant, disodium salt of ethylene diaminetetra-acetic acid (EDTA) at the rate of 2 mg/ml of blood (18), while the remaining blood was immediately centrifuged 3,500 rpm for 15 minutes and the resultant serum harvested and stored in the refrigerator until needed for analysis. Haematological parameters determined included; Packed Cell Volume (PCV) total red blood cell (RBC) counts and haemoglobin concentration as described by (19) as well as the erythrocyte osmotic fragility (EOF) using the method of (20). Blood smears were made for subsequent differential leukocyte analysis following May-Grunwald staining (21) and H/L ratios were calculated (22).

The stored serum was used to assay for Cortisol determination using ELISA quantitative (Diagnostic Automation, Inc.) method, Serum malondialdehyde (MDA) concentration as a marker of lipid peroxidation was determined by the double-heating method of (23) as modified by (24). Alkaline phosphatase, alanine amino transferase (ALT), aspartate aminotransferase (AST), serum albumin, total cholesterol, urea were all determined as described by (25).

Statistical Analysis

Data were subjected to Student's *t*-test using Graph Pad Prism version 4.00 for Windows and the data are expressed as mean ± standard error of the mean (mean ± SEM). Values of $P<0.05$ were considered significant.

RESULTS AND DISCUSSION

The mean maximum ambient (AT) temperature of 37.95 ± 0.65°C and minimum AT of 26.35 ± 0.50°C was obtained during the study period with a maximum relative humidity (RH) of 62.00 ± 5.00% and minimum of 36.50 ± 7.50%. A higher significant ($P<0.05$) value of cortisol and MDA concentration of 1.17 ± 0.01 ng/ml and 2.18 ± 0.22 ng/ml was obtained in control group respectively as against 0.02 ng/ml 0.93 ± 0.09 ng/ml of cortisol and MDA recorded in the experimental group respectively. The values recorded for catalase activity (62.25 ± 5.12 u/ml), superoxide dismutase (3.20 ± 0.20 u/ml) and glutathione peroxidase (49.00 ± 3.56 u/ml) in the control group was significantly ($P<0.05$) higher than the corresponding values in experimental group catalase (51.75 ± 1.43 u/ml), superoxide dismutase (2.18 ± 0.03 u/ml) and glutathione peroxidase (46.00 ± 1.19 u/ml). The obtained value of alkaline phosphatase in the control animal group was significantly ($P<0.05$) higher than the corresponding value in experimental group (Table 1). The PCV value of 24.25 ± 2.80% obtained in the control group was significantly ($P<0.05$) lower than 31.50 ± 13.13 % recorded in the experimental group. The haemoglobin concentration (Table 2) total erythrocytes count were significantly ($P<0.05$) lower in the control group than the experimental group. The obtained total leukocytes count in the control group (3.80 ± 0.05 x 10^3/l) were significantly ($P<0.05$) higher than that of experimental birds (3.40 ± 0.20 x 10^3/µl)**,** however value of 40.82 ± 0.20% of heterophils obtained in the control group was significantly ($P<0.05$) less than 31.90 ± 0.30% recorded in the experimental animal. The obtained lymphocyte value of 50.45 ± 0.09% in the control group was significantly ($P<0.05$) lower than 57.50 ± 0.03% recorded in experimental group. The Heterophils/Lymphocytes value of 0.55 recorded in the experimental group was significantly ($P<0.05$) lower than 0.81 seen in the control group. The minimum heamolysis was obtained at 0.85% sodium chloride concentration in the experiment group with a value of 1.43 ± 0.01% while the corresponding value in the control group was 10.98 ± 0.05% and this value was significantly ($P<0.05$) different. At 0.5% sodium chloride concentration, a higher significant ($P<0.05$) value of haemolysis was recorded in the control group (76.18 ± 2.50%) while a corresponding value of 27.84 ± 1.02% was obtained in the experimental group.

Table 1. Effect of Ascorbic acid Administration in Drinking water on Serum Biochemical and Oxidative Stress Parametersof Control and Experimental of Broiler chicken (Mean ± SEM)

Parameters	Control	Experimental
Cortisol (ng/ml)	1.17 ± 0.01[a]	2.18 ± 0.02[b]
Serum Malondialdehyde (ng/ml)	2.18 ± 0.22[a]	0.93 ± 0.09[b]
Superoxide dismutase (u/ml)	3.20 ± 0.20[a]	2.18 ± 0.03[b]
Catalase (u/ml)	62.25 ± 5.12[a]	51.75 ± 1.43[b]
Glutathione peroxidase (u/ml)	49.00 ± 3.56[a]	46.00 ± 1.19[b]
Aspartate amino transferase (I.U./L)	42.75 ± 3.30[a]	36.45 ± 1.54[b]
Alanine amino transferase (I.U./L)	49.00 ± 6.48[a]	44.50 ± 1.09[b]
Alkaline phosphatase (I.U./L)	71.5 ± 1.15[a]	58.30 ± 1.45[b]
Urea (mg/100ml)	2.58 ± 0.54	2.75 ± 0.21
Total Cholesterol (mg/100ml)	2.10 ± 0.18	1.83 ± 0.23

Means within row with different superscripts are significantly (P<0.05) different.

Table 2. Effect of Ascorbic acid Administration in Drinking water on Haematological Parameters of Control and Experimental of Broiler chicken (Mean ± SEM)

Parameters	Control	Experimental
Packed Cell Volume (%)	24.25 ± 2.80[a]	31.50 ± 13.13[b]
Haemoglobin Concentration (gm/dl)	8.08 ± 0.83[a]	10.50 ± 4.38[b]
Total Erythrocytes Count (x 10^6/µl)	4.35 ± 1.13[a]	5.94 ± 1.28[b]
Total Leukocytes Count (x 10^3/µl)	3.80 ± 0.05	3.40 ± 0.20[a]
Heterophils (%)	40.82 ± 0.20	31.90 ± 0.30[a]
Lymphocytes (%)	50.45 ± 0.09[a]	57.50 ± 0.03[b]
Monocytes (%)	4.70 ± 0.25[a]	2.60 ± 0.02[b]
Basophils (%)	1.7 0 ± 0.01	1.40 ± 0.01
Eosinophils (%)	2.50 ± 0.07	6.20 ± 2.20
Heterophils/Lymphocytes (%)	0.81[a]	0.55[b]

Means within row with different superscripts are significantly (P<0.05) different.

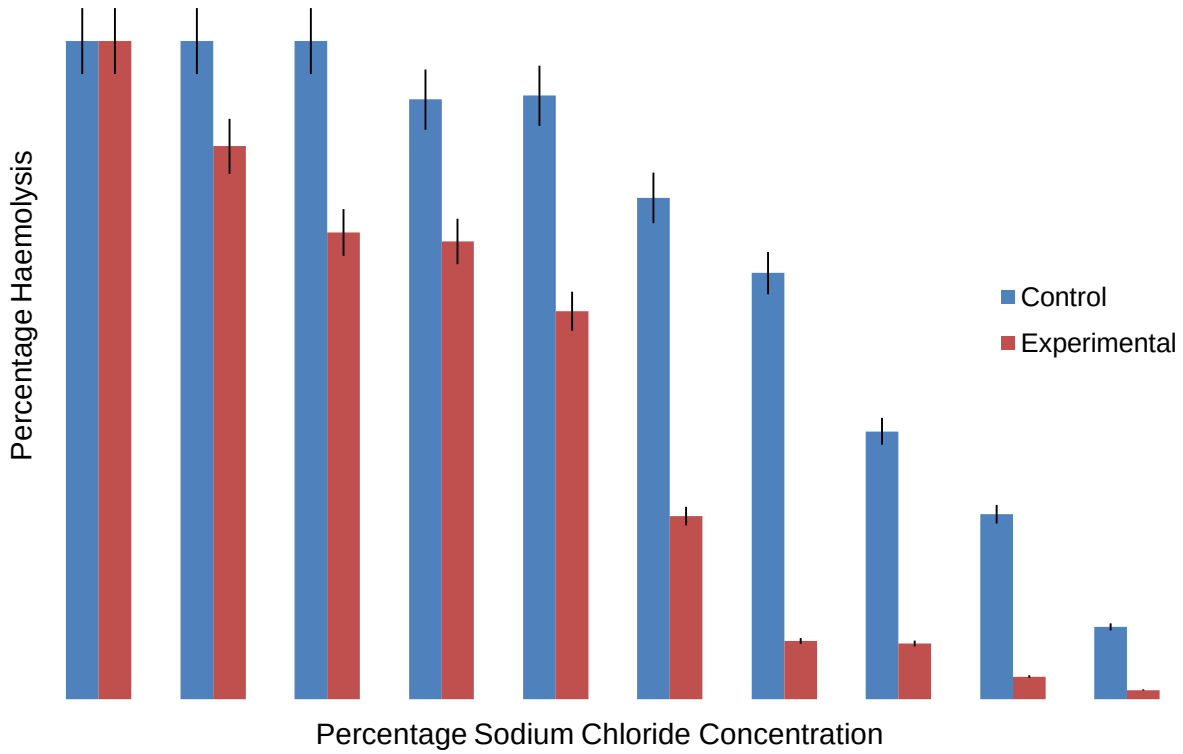

Figure 1. Effect of Ascorbic acid Administration in Drinking water on Erythrocyte Osmotic Fragility of Control and Experimental Broiler Chicken (Mean ± SEM)

The result of the present study indicated that the chicken were exposed to ambient temperature of 37.95 ± 0.65°C – 26.35 ± 0.50°C predominantly outside the thermoneutral zone of 12 – 24°C (27) for birds in the tropics and 18 – 24°C for birds in the tropics (14) and as such this season is thermally stressful and may have adverse effect on their homeostatic mechanism. It has been documented that changes in thermal environment caused by fluctuations in AT and RH induce a variety of physiological responses (15). The finding of this study agrees with the work of (28) who reported that a high temperature, accompanied by high humidity, is more detrimental to bird performance than high temperature with low humidity. Similarly, the results of this work is in agreement with the findings of (29) and (30) who reported that the hot-dry season is thermally stressful to animals. During heat stress the natural antioxidants responsible for eliminating harmful free radicals are overwhelmed or exhausted and also the body requirements of ascorbic acid during heat stress in poultry is greater than the amount synthesized by normal tissues and its administration to birds during heat stress has been shown to be beneficial to the body (15, 40). High AT stimulates the hypothalamus via the hypophyseal-adrenocortical axis which increases cortico-steroid secretion in response to stress (31). Higher levels of circulating corticosteroids have a catabolic effect through increase in the free radicals generation (32) as evidenced by higher values of cortisol and that of serum MDA in the control group than experimental group. MDA has been shown to be a biomarker of lipid peroxidation (33) which is the oxidative deterioration of polyunsaturated fatty acids, in the cell membranes, which leads to a sequential milieu of chemical reactions (34, 35) as a result of enormous generation of reactive oxygen species (free radicals) generated during heat stress (34, 35). It has been well documented that free radicals is a compound carrying an unpaired electron, induces chain reactions with another compound to generate an unpaired electron, such that radical begets radical. These reactions are accomplished through three main steps, namely, initiation, propagation, and termination (36, 37). The adverse physiological reactions, culminating in lipid peroxidation in cytomembranes induced by free radicals generated during heat stress have been reported in avian species (38, 39) and supplementation of AA alleviate the adverse effects of heat stress in broiler chickens in this study by reducing the biomarker of lipid peroxidation (MDA concentration), AA has also been documented to strongly reduce MDA concentration in erythrocyte of chickens (40). AA is a potent antioxidant compound in the mitigation of and prevention of adverse effects of stress in livestock (5). Poultry can synthesize AA but its quantity is insufficient for reduction of free radicals (13) because body requirements of AA during heat stress in poultry is greater than the amount synthesized by normal tissues and its administration to broilers during heat stress has been shown to be beneficial to the body (41, 42). AA as antioxidants donates free molecules of hydrogen that detoxify the harmful reactive oxygen species, especially when the natural antioxidants in the body are exhausted or overwhelmed (43).

The results of this study have shown consistency with previous experiments as reported by (44) that the cellular anti-oxidants defence system consists of superoxide dismutase, catalase and glutathione peroxidase. The shift of the delicate balance between free radicals and cellular antioxidants defence system in favour of free radicals might lead to development of oxidative stress. As a result of heat stress, the amount of free radicals increase in the body and this leads to defence system to start producing these enzymes. This could probably account for high value of these enzymes in the control group while AA being potent antioxidants could possibly have scavenged for these free radicals in the experimental group.

The ameliorative action of AA was also noted in transaminase enzymes where their levels were optimized as compared to the control. The finding is in agreement with the earlier work of (45) who noted a decrease in the values of these enzymes after supplementing layers with vitamin C. The finding is also in corroboration with that of (32) where polyherbal premix (Stressroak at 1 kg/tone of feed) was used to minimize heat stress in broiler during summer months. The higher value seen in the control group could be as a result of assault of free radical generation on the liver brought about by heat stress, but this was ameliorated in the experimental group with AA supplementation.

The lower value of packed cell volume, haemoglobin concentration and total erythrocytre count obtained in the control group may be attributed to the ability of AA to maintain the integrity of erythrocyte membrane in AA-treated group (46) and also been able to scavenge for enormous amount of free radicals which is more in the control group as indicated by high MDA concentration. The results of this study suggested that AA prevented the release of leucocytes from their pool in the body into peripheral circulation, apparently due to its inhibitory role on circulating corticosteroids in animals under stress, possibly that is why there is high value of leucocyte in the control group compared to the experimental group. Chronic heat stress caused changes in the proportion of leukocyte (47). It has been reported that after 2 hours heat stress, broiler chickens exhibited raised heterophils and significantly reduced lymphocytes (48). lymphocyte and raised heterophils. As a consequence, the heterophils/lymphocyte ratio (H/L) increased due to heat stress, is in agreement with studies of (49) and (9). The H/L ratio has been used as a reliable indicator of stress in pigs (5) and in birds (50), indicating that, in our study, birds from the control group were significantly stressed compared to treated groups.

Erythrocyte osmotic fragility (EOF) has been shown to be related to its geometric configuration of the erythrocyte (Figure 1), which in turn depends on the integrity of the cell membrane (19). The compromise of the erythrocyte membrane integrity resulting in increased EOF may have been caused by increased lipoperoxidative changes which lead to the destruction of erythrocytes which is more in control group than the experimental group as seen in this study evidenced by increase in haemolysis of the control group. The oxidative modification of the erythrocyte membrane caused by increase radical generation as a result of heat stress (6) has been shown to increase the fragility of the RBC (51). The results of this study agree with the findings of (8) and (9) who showed that free radicals play a vital role in tissue damage and have adverse effects on erythrocytes.

In conclusion AA administration mitigate the adverse effects brought about by heat stress in broiler chicken; its supplementation is thus recommended during the hot-dry season to alleviate heat stress.

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