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Plasma corticosterone and adrenal gland histomorphometry of heat stressed broiler chickens given supplemental electrolytes or vitamin C

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Additional keywords

Adrenal gland histomorphometry.
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SUMMARY

The effect of oral electrolytes or vitamin C (VC) on plasma corticosterone (PC) and adrenal gland (AG) histomorphology of heat stressed broiler chickens was evaluated in this study. A total of 200 one day-old Arbor Acre broiler chickens were allotted to five treatments in a completely randomized design. Each treatment comprised four replicates of ten chickens per replicate. Treatment 1 (T1) was the control with water without any supplement while treatments 2 (T2), 3 (T3), 4 (T4) and 5 (T5) had their water supplemented with 0.5% ammonium chloride, sodium bicarbonate, calcium chloride and 300ppm VC, respectively. The average temperature and humidity during the experimental period ranged from 30.90°C to 36.73°C and 58.48% to 89.24% respectively. No significant variation (p>0.05) was observed in PC values among treatments at week 4. However, PC (ng/mL) values of 6.1±1.3 (control), 7.4±2.1 (NH_4Cl), 5.4±0.8 ($NaHCO_3$), 15.1±5.91 ($CaCl_2$) and 6.4±2.4 (VC) recorded at week 7 significantly differed (p<0.05). Birds on VC supplementation had increased medullary cells (0.59/µm^3 x 10-6 ± 0.12) and lowered interrenal cells (0.36/µm^3 x 10-6 ± 0.18) compared to birds on other treatments. Histological changes observed in the AG of birds on control and electrolyte treatment groups revealed evidence of stress at the cellular level while a relatively normal cell appearance was observed in VC group. In conclusion, the reduction in the PC values of the ammonium chloride and sodium bicarbonate groups was similar to the observations from the control group. However, ascorbic acid maintained corticosterone concentration and prevented from the hyperplasia of interrenal cells in heat stressed birds.

Plasma histomorphométrie corticostérone et glande surrénale de chaleur a souligné des poulets de chair données électrolytes supplémentaires ou vitamine C

Mots-clés supplémentaires

Glande surrénale histomorphométrie.
Chaleur a insisté sur les poulets de chair.
Corticostérone plasmatique.
Vitamines supplémentaires et d'électrolyte.

RÉSUMÉ

L'effet des électrolytes par voie orale ou vitamine C (VC) sur la corticostérone plasmatique (PC) et de la glande surrénale (AG) histomorphologie de la chaleur des poulets soumis à une contrainte a été évalué dans cette étude. Un total de 200 un jour-vieux Arbor Acres poulets de chair ont été attribuées à cinq traitements dans un design complètement randomisé. Chaque traitement comprenait quatre réplicats de dix poulets par réplicat. Traitement 1 (T1) était le contrôle avec de l'eau sans aucun supplément lors de traitements 2 (T2), 3 (T3), 4 (T4) et 5 (T5) avaient leur eau additionné de 0,5% chlorure d'ammonium, bicarbonate de sodium, chlorure de calcium et 300ppm VC, respectivement. Pas de variation significative (p>0,05) a été observée dans les valeurs de PC entre les traitements à la semaine 4. Toutefois, les valeurs de la PC (ng/mL), de 6.1±1.3 (contrôle), 7.4±2.1 (NH_4Cl), 5.4±0.8 ($NaHCO_3$), 15.1±5.91 ($CaCl_2$) et 6.4±2.4 (VC), enregistrées à la semaine 7 diffèrent significativement (p<0,05). Les oiseaux sur la supplémentation en VC avaient accru de cellules médullaires (0,59/µm^3 x 10-6 ± 0,12) et abaissé les cellules interrénales (0,36/µm^3 x 10-6 ± 0,18) par rapport aux oiseaux sur d'autres traitements. Modifications histologiques observées dans le AG d'oiseaux sur le contrôle et les groupes de traitement électrolytique a révélé des preuves de stress au niveau cellulaire tout en apparence relativement normale de la cellule a été observée dans le groupe de VC. En conclusion, réduction des valeurs de PC des groupes chlorure d'ammonium et bicarbonate de sodium a été similaire à l'observation pour le groupe témoin. Cependant, VC maintient la concentration de corticostérone et prévient aussi l'hyperplasie des cellules interrénal en chaleur a insisté sur les oiseaux.

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INTRODUCTION

Activation of the hypothalamic-pituitary-adrenal (HPA) axis is a major neuroendocine mechanism in a stress reaction (Axelrod and Reisine, 1984). This result in a rapid increase in circulating corticotrophin (ACTH) and subsequent rise in glucocorticoids which according to Dallman *et al*. (1992) are critical for successful adaptation. However, as a result of the HPA axis stimulation, significant changes occur in the adre-

nals. Nussdorfer (1986) reported increase in adrenal mass and steroidogenic capacity of adrenocortical cells as response to long term effect of ACTH secretion. One of the hormonal responses to heat stress is an increase in blood concentrations of corticosterone, the primary glucocorticoid hormone produced by the avian adrenal gland. Studies have linked increased levels of blood corticosterone with changes in glucose, and mineral metabolism, cardiovascular diseases, hypercholesterolemia, gastro intestinal lesions and alteration in immune system function (Siegel *et al*., 1995; Puvadolpirod and Thaxton, 2000).

As distinct from the mammalian adrenal gland, the cortical or interrenal tissue of avian adrenal gland intermingles with the medullary or chromaffin tissue and their distribution throughout the gland is said to be uniform, although the ratio of one type of tissue to the other varies (Wells and Wight, 1971). The medullary tissue has been consistently said to have a higher percentage volume than the cortical tissue in the adult (Sivaram, 1964), but this ratio varies with age, sex, health and environmental factors (Oakberg, 1951). Wells and Wight (1971) therefore considered the determination of the normal structure and distribution of the two component tissue types to be of great value as a base-line for physiological and pathological evaluation of the avian adrenal gland.

Ascorbic acid supplementation improved performance of heat challenged broiler chickens and has been associated with lower plasma corticosterone concentrations (Kutlu and Forbes, 1993; Mckee and Harrison, 1995). Better performance and reduced heat stress related mortality in broiler chickens given ascorbic acid and electrolytes have also been widely reported (Pardue *et al*., 1985; Ogunwole *et al*., 2013; Majekodunmi *et al*., 2015). However, there has been dearth of information on the histomorphometric response of adrenal gland of heat stressed broilers in hot humid tropics. Therefore, this study was aimed at determining the possible effect of administering supplemental oral electrolytes or vitamin C on blood corticosterone and adrenal gland histomorphology of broiler birds reared during hot period in the hot humid tropics of Nigeria.

MATERIALS AND METHODS

Experimental birds and management

The study was carried out at the Teaching and Research Farm of the University of Ibadan, Ibadan which is located between latitudes 6°10″ and 9°10″ North of the equator and longitudes 30° and 60° of the Greenwich for a period of seven weeks. A total of 200 one day-old Arbor Acre broiler chicken strain were allotted to five treatment groups. Each treatment was replicated four times with 10 birds per replicate in a completely randomized design (CRD). Formulated broiler starter and finishers' diets which contained 3000 KCal/Kg ME and 23 % CP; 3000 KCal/Kg ME and 19 % CP respectively. Detailed gross composition of the experimental diets has been documented (Majekodunmi *et al*., 2012; 2013) and is shown in **table I**. Experimental feed was offered to birds ad libitum throughout the experimental period.

Clean water in which test electrolytes or vitamin C has been added was also provided ad libitum. The treatments were: Treatment 1(control) was without any electrolyte or vitamin, Treatment 2 (0.5% ammonium chloride), Treatment 3 (0.5% sodium bicarbonate), Treatment 4 (0.5% calcium chloride), and Treatment 5 (300ppm vitamin C). The experiment was carried out during the hot period of the year (March- April) and average temperature and humidity recorded during the experimental period ranged from 30.90°C to 36.73°C and 58.48% to 89.24% respectively.

Blood collection

Blood samples were collected between 6:30 and 7:30 am from twelve randomly selected birds in each treatment at the end of week 4 and 7 of the experiment. Birds were bled through the jugular vein and blood was collected into vaccutainers tube containing EDTA, centrifuged at 1500 x g for 10minutes and the plasma was decanted and stored at -20°C for corticosterone measurement. In order to minimize stress induced procedure on the birds, it was ensured that each bird was caught and bled in less than one minute.

Plasma corticosterone assay

Plasma corticosterone level was determined by Enzyme-Linked ImmunoSorbent Assay (ELISA) using a commercial kit. All samples were run in duplicate and absorbance was measured at 650nm in an ELISA micro plate reader.

Histological analysis

Five birds were randomly selected from each replicate and sacrificed. Adrenal glands were removed and weighed. The left adrenal glands were fixed in Bouin's fluid, embedded in paraffin wax, cut at 5 microns and stained with haematoxylin and eosin, and Masson's trichrome stain. Five sections, representative of each adrenal gland, were studied as described (Gray, 1996).

Numerical density was calculated by dividing the total number of cells counted in all dissectors in an adrenal gland by the cumulative volume of the dissectors (area of the counting frame multiply by dissector height) sampled in the adrenal gland (Bielohuby *et al*., 2007).

Statistical design

All data were subjected to analysis of variance (SAS, 1999). Treatment means were compared using the Duncan's option of the same software.

RESULTS

Plasma corticosterone and numerical density of adrenal gland cells

Plasma corticosterone (PC) concentration of heat stressed broiler birds given water supplemented with electrolytes and ascorbic acid is shown in **figure 1.** No significant difference ($p>0.05$) was observed in plasma corticosterone concentration among treatments in week 4. The values ranged from 4.89-12.50 ng/mL with birds on treatment 3 (sodium bicarbonate) having the highest concentration (12.50 ng/mL) while birds on

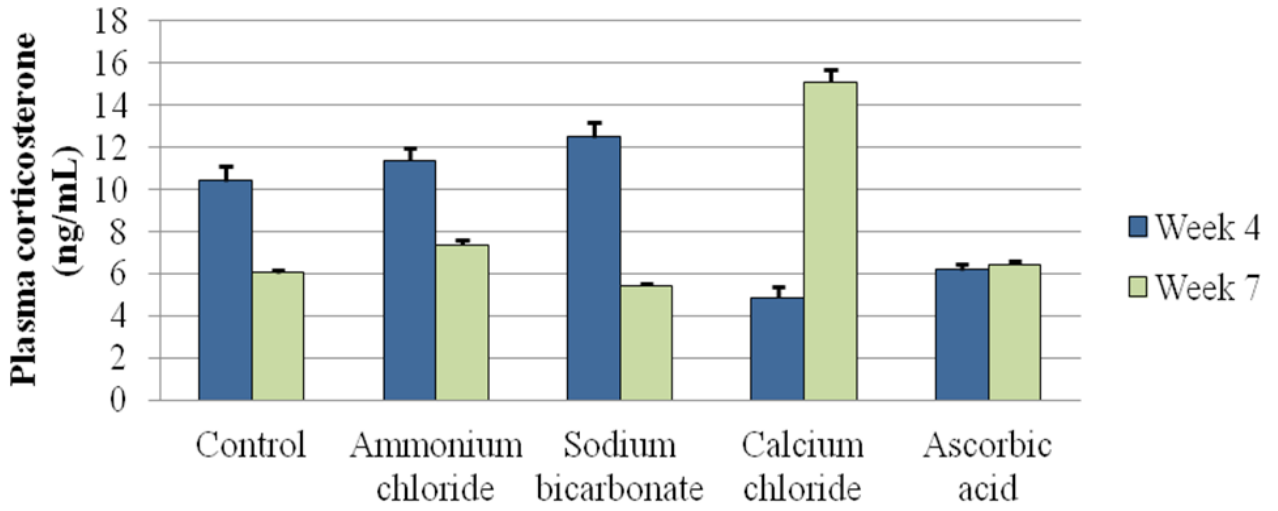

Figure 1. Plasma corticosterone of heat stressed broiler chickens provided with electrolytes or ascorbic acid supplemented water (Corticostérone plasmatique de chaleur a souligné poulets à l'eau additionnée d'électrolytes ou acide ascorbique)

treatment 1 (control), treatment 2 (ammonium chloride), treatment 4 (calcium chloride) and treatment 5 (ascorbic acid) had PC values of 10.43 ng/mL, 11.38 ng/mL, 4.88 ng/mL and 6.20 ng/mL respectively. However, at week 7 birds given water supplemented with calcium chloride had significantly higher ($p<0.05$) PC concentration of 15.10 ng/mL compared with birds on treatments 1 (6.05 ng/mL), 2 (7.38 ng/mL), 3 (5.43 ng/mL) and 5 (6.43 ng/mL) .There were therefore decrease in the corticosterone concentration in birds on treatments 1, 2 and 3 which were 4.38, 4.00, and 7.07ng/mL respectively. Whereas, there was about 3-fold increase (10.21ng/mL) in the corticosterone concentration of birds on treatment 4 while there was marginal increase in those from treatment 5 (0.23 ng/mL) over week 4 value.

Numerical density (Nd) of cells in the cortex and medulla of adrenal glands of heat stressed birds given water supplemented with electrolytes and ascorbic acid is shown in **figure 2**. Significant variations ($p<0.05$) were observed in the Nd of both interrenal and medullary cells among treatments. The highest interrenal density was observed in birds on treatment 1($1.05/\mu m^3 \times 10^{-6}$) which was significantly different ($p<0.05$) from the values reported for birds on treatments 2, 3 and 5 which were $0.63/\mu m^3 \times 10^{-6}$, $0.50/\mu m^3 \times 10^{-6}$ and $0.36/\mu m^3 \times 10^{-6}$, respectively. Similarly, Nd of medullary cell, also recorded a significantly higher ($p<0.05$) value ($0.89/\mu m^3 \times 10^{-6}$) for birds on treatment 1 compared with birds on treatment 3 ($0.38/\mu m^3 \times 10^{-6}$).

Figure 3 shows the microphotographs of adrenal gland of broiler birds given water supplemented with

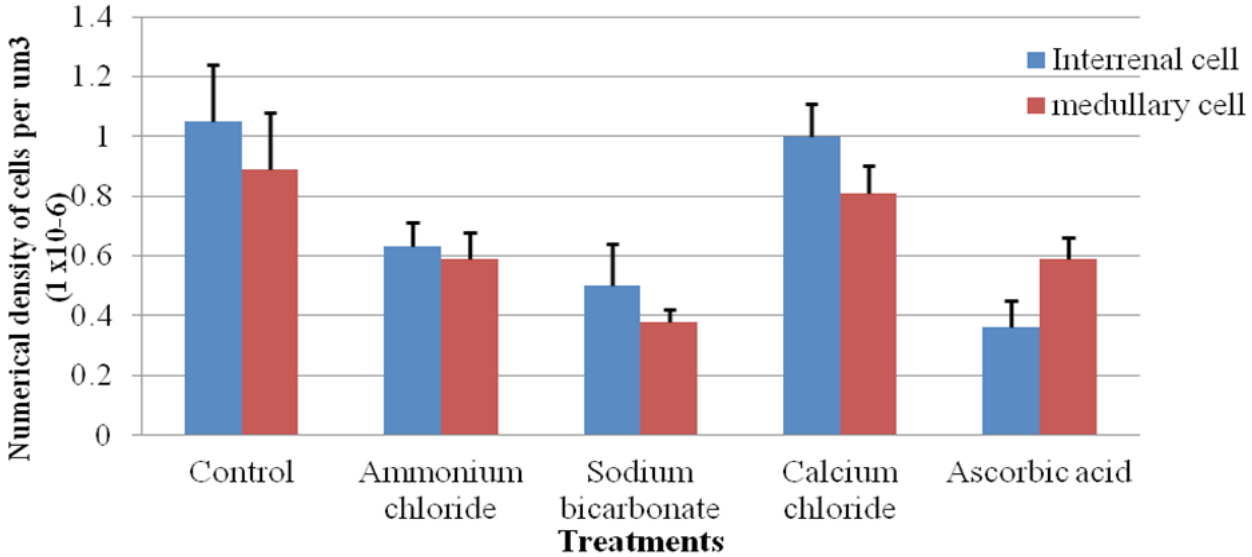

Figure 2. Numerical density of cells in the cortex and medulla of adrenal glands of heat stressed birds provided with electrolytes or ascorbic acid supplemented water (Densité en nombre de cellules dans le cortex et la médulle des glandes surrénales d'oiseaux affectée par la chaleur complété avec des électrolytes ou de l'acide ascorbique dans l'eau)

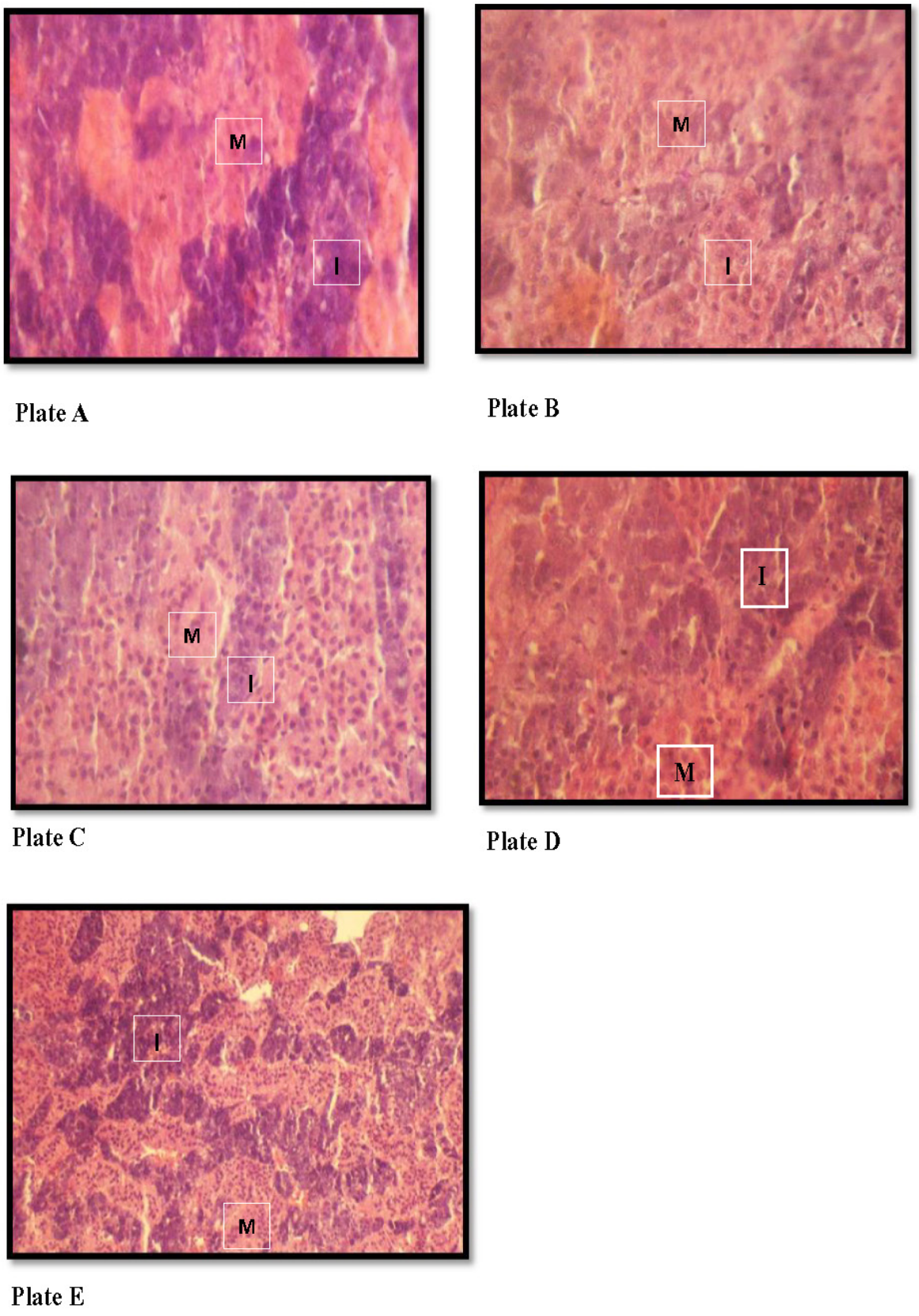

Figure 3. Microphotographs of the adrenal gland of broiler birds: A-control group (T1), B-Ammonium chloride (T2), C-Sodium bicarbonate (T3), D-Calcium chloride (T4) and E-Ascorbic acid (T5) (Photomicrographie de glande surrénale des oiseaux de poulets de chair : groupe A-témoin (T1), chlorure d'Ammonium B (T2), C-bicarbonate de soude (T3), chlorure de D-Calcium (T4) et E-ascorbique acide (T5) M-médullaires des cellules, j'ai-interrénal) M- Medullary cells, I- Interrenal cells)

electrolytes and ascorbic acid. Plate A of **figure 3** shows faint cells with blurred cellular outlines. Plate B revealed swollen chromaffin cells with expanded cytoplasm. Plate C shows extensive cellular necrosis of both areas. Plate D shows blurred cellular outlines with necrosis. Plate E shows relatively normal cell appearance.

DISCUSSION

The reduction observed in the corticosterone concentration of birds on all the treatments except treatment 4 (calcium chloride) at week 7 in this study might be that the stress period was longer and the birds were beginning to acclimate to the stressor which agreed with the findings of Thaxton *et al*. (2005) of a consistent decrease in blood corticosterone concentration from week 2 to week 7. This further suggested an apparent physiological adaptation of broilers to their environment which is enhanced as the birds approached market age. It has been demonstrated (Freeman, 1970; Puvadolpirod and Thaxton, 2000) that corticosterone secretion increased at high temperatures. Kutlu and Forbes (1993) reported that ascorbic acid induced a significant reduction of the glucocorticoid synthesis in birds. Similarly, Sahin *et al*. (2002) reported low plasma concentrations of ACTH in quail reared at 32°C and supplemented with vitamin C. The proposed mechanism for this effect is through inhibition of the activity of the hydroxylase enzymes (21-hydroxylase and 11-beta hydroxylase) by ascorbic acid in the steroid (corticosterone) biosynthetic pathway. The increased PC concentration in calcium chloride group (treatment 4) at week 7 may indicate the potential of calcium chloride in inducing abnormal metabolic acid-base balance in heat stressed birds. Calcium chloride is mainly excreted in the faeces as calcium carbonate, the chloride replacing bicarbonate in the body causing acidosis. Bottje and Harrison (1984) reported decrease in blood bicarbonate in calcium chloride infused birds which according to them demonstrates an overriding metabolic acidosis during heat stress.

The adrenal medulla in mammalian species is surrounded by a cortex that contains three distinct layers, whereas the cortex and the medulla are intermingled in poultry species (Basha *et al*., 2009). In birds, the adrenal gland is characterized by the presence of cortical or inter-renal tissue which intermingles with the medullar or chromaffin tissue with a relatively uniform distribution but the ratio of one type of tissue to the other remains variable (Well and Wight, 1971). The relative volume proportions of the medullar tissue are usually considered to be higher than those of the cortical tissue in adult but this ratio varies with age, sex, health and environmental factors (Well and Wight, 1971). The adrenal glands are known to respond to stress conditions, particularly environmental conditions, by interrenal hyperplasia in birds (Freeman, 1970). Observed elevation in PC levels of control, ammonium chloride and sodium bicarbonate groups at week 4 and calcium chloride at week 7 could explain the relatively abnormal cellular appearances in the interrenal and medulary cells of the adrenal glands of birds in these groups. However, the eventual reduction in PC values of birds in control, ammonium chloride and sodium bicarbonate groups could be as a result of natural adaptation responses of the birds to heat stress.

In this study, vitamin C supplementation prevented hyperplasia of interrenal cell which was evident in the significant reduction in the numerical density of the interrenal cell responsible for the corticosterone synthesis in birds supplemented with vitamin C compared with other treatment groups. This observation conformed to earlier report (Ozdemir *et al*., 2009) of increased proportion of medullar zone in birds given diet supplemented with vitamin C. Histological changes observed for birds on control and electrolyte treatment groups revealed evidence of stress at the cellular level while the relatively normal cell appearance observed in ascorbic acid group could be attributed to the antioxidant effect of vitamin C in protecting the adrenal gland cells. This observation perhaps indicated that birds on treatment 5 (ascorbic acid) were relatively more comfortable and less stressed compared with birds on other treatments. This effect was considered as beneficial for birds under heat stress.

CONCLUSION

The reduction in plasma corticosterone values recorded in the ammonium chloride and sodium bicarbonate groups was not different from the observation for the control birds. Whereas, ascorbic acid was able to mitigate excessive production of corticosterone expected during heat stress and also prevented proliferation (hyperplasia) of interrenal cell of stressed broiler chickens.

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