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Modulatory Effect of Ascorbic Acid Supplementation on the Physiological and Behavioural Parameters in West African Dwarf Goats Confined During the Rainy Season

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SUMMARY

This study was carried out with the aim of investigating the modulatory effect of ascorbic acid (AA) on the physiological and behavioural parameters in confined West African dwarf goats during the rainy season. A total of fourteen adult West Africa Dwarf goats, male and non-pregnant, non-nursing female were used for the study. The animals were divided into two groups of seven animals each, experimental for those in group 1 and control for group 2. The haematological parameters obtained in experimental animal pre-confinement was not significantly ($P > 0.05$) different from the values obtained in the control animal during the same period. The values of leukocyte (7.57 ± 0.37 x 103/µl) obtained in the experimental animal post-confinement was significantly ($P < 0.05$) lower than 9.23 ± 0.85 x 103/µl obtained in the control animal. There was no significant ($P > 0.05$) difference in the recorded haemolysis between experimental and control animals at pre-confinement. The lowest percentage haemolysis of 1.58 ± 0.62 % was obtained in experimental animals post confinement at 0.85 % of Sodium Chloride concentration while the corresponding value in the control animals was significantly ($P < 0.05$) higher. The excitability score in experimental animals with a value of 42.86 % was significantly ($P < 0.05$) higher than the control animals with a value of 0.00 % obtained during confinement. The same trend was recorded post confinement. All the observed behavioural activities pre-confinement was not significantly ($P > 0.05$) different in both experimental and control goats. The behavioural activities of standing (71.43 %) in experimental animals was significant ($P > 0.05$) higher than the value of 42.86 % in control animals. Also the behavioural activities of eating and drinking with a value of 71.43 % and 57.14 % respectively was significantly ($P < 0.05$) greater in experimental animals than the corresponding value of 28.57 % and 14.29 % in the control animal respectively while a value of 57.14 % recorded for behavioural activities of lying down in control animals was greater than 28.57 % recorded in experimental animals during confinement. The behavioural activities of standing (85.71 %), sniffing (57.14 %), fighting (57.14 %), eating (71.43 %) and drinking (57.14 %) was significantly ($P < 0.05$) higher in experimental animals compared to the corresponding values in the control animals post-confinement. In conclusion, it is thus recommended that ascorbic acid should be administered to goats in confinement during rainy season to ameliorate the stress induced by confinement as this may enhance their health and productivity during this period.

INTRODUCTION

In Nigeria management of goat is largely in traditional hands (Ajala, 1995) where they are allowed to roam freely around their environment to scavenge for food scrap and crop residue. The systems of management are dictated by climate, cropping and population density. Confinement is a management practice used in Nigeria especially in the rainy season by tethering the animal to prevent crop damage (Adu and Ngere, 1979; Okello and Obwolo, 1985; Neil et al., 2009). This practice predisposes animals to some stress factors (Ayo and Minka, 2003; Adenkola and Ayo, 2006) which act on them concomitantly. Factors like physical discomfort, behavioural deprivation and emotional reaction (Ademosun, 1987, Danilevsky, 1991) constitute environmental stress on this animal and this stress in turn causes oxidative stress which impairs antioxidant status in vivo (Sahin et al., 2001). Thus there is therefore a need to administer an antioxidant exogenously to boost endogenous source especially during stressful situation. Ascorbic acid (AA) has been reported to increase body resistance to environmental stress (Tauler et al., 2003) by reducing the synthesis and secretion of corticosteroids thus alleviating the negative effect of stress. AA is a water-soluble antioxidant vitamin, non-toxic, sustainable and readily metabolized by the body of most domestic animals and humans (Hickey et al., 2008) and is affordable and readily available.

The aim of this experiment is to determine the modulatory effect of ascorbic acid on the physiological and behavioural parameters in confined West African dwarf goats

MATERIALS AND METHODS

Experimental Site

The study was conducted at Small Ruminant Unit of the University of Agriculture Teaching and Research Farm Makurdi (070 41/ N, 080 37/ E) in the Southern Guinea Savannah Zone of Nigeria. The area has a very warm weather with daily mean temperature ranging from 26.5 – 42.00 C, with an annual rainfall of 1,317 – 1,323 mm which span between 6 – 7 months (Adenkola et al., 2010).

Meteorological Data

The meteorological data for the study period which included the minimum and maximum ambient temperature (AT), relative humidity (RH), rainfall, sunshine hour per day, wind speed and wind direction for the period of study was obtained from the Nigerian Meteorological Agency (NIMET) Makurdi.

Experimental Design

A total of fourteen adult West Africa Dwarf goats, male and non-pregnant, non-nursing female aged 1.0 – 1.5 years, and weighing 9.5 ± 0.29 kg were used for the study. The animals were divided into two groups of seven animal each tagged experimental for those in group 1 and control for animals in group 2. The animals were kept in one of the pens in the building meant for small ruminant in the farm. The house in which the animals were kept had a long corridor of about 1m long with each pen measuring 285 x 285 cm for animals on both sides of the corridor. The pen had a large wide windows measuring 180 x 126 cm with a wire mesh for natural ventilation. Prior to the commencement of the experiment the animals were kept for 2 weeks in the same pen but they were allowed to graze during the day between the hours of 9:00 hr to 18:00 hr in the evening before moving them to experimental pen. Also during this period they were screened for possible endo and haemo-parasites and were treated accordingly with 20 mg/kg of oxytetracycline (20 %) (Tridox®, Farvet Laboratories, Handelsweg, Holland). On the experimental day which is the day of confinement, the experimental goats each were administered with 250 mg/kg of ascorbic acid (Juhel Nigeria limited) dissolved in 20 ml of water while control goats were given 20 ml of water only per head. During confinement, water was provided for the goats ad libitum and were also fed ad libitum with grass comprising

of elephant grass (Panicum maximum), gamba grass (Andropogon gayanus) and legumes (mainly Centrosema spp.) without any supplementary feeding, cut from the natural pasture around the University of Agriculture Teaching and Research Farm premises where the animals were grazing before confinement. The animals were confined for a period of fourteen days in a pen in the farm. Blood samples were collected early in the morning a day before and on day 14 of confinement, immediately after which the experiment was terminated. Blood sample (3 ml) was collected aseptically via the jugular vein from each animal and this 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. After collection, the samples were transferred to Physiology Laboratory, Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Agriculture, Makurdi, where they were analyzed for packed cell volume (PCV) using microhaematocrit method, total erythrocyte count, total leucocyte count using haemocytometric method and Haemoglobin (Hb) concentration as described by Schalm et al. (1975). Erythrocyte osmotic fragility was also determined as described by Faulkner and King (1970).

Measurement of Rectal Temperature

Briefly the rectal temperature (RT) was measured using a standard thermometer (Harman digital thermometer, Germany), inserted approximately 5-6 cm through the anus into the rectum of each
goat and left there for 5 minutes after which the value was read as correspond to the level of the rising mercury in the thermometer indicating the end of the reading (Adenkola and Ayo, 2009b).

Measurement of Excitability Score and Body weight

The live weight of each WAD goat was measured using standard weighing scale (Sunbeam Coy, USA) at 07:00 h on day 1, 7 and 14 of confinement. Excitability scores were recorded during weighing of each goat as described by Voisinet et al. (1997) and Kannan et al. (2002).

Investigation of Behavioural Activities of Goat

The behavioural activities of goat which included standing, lying down, sniffing, fight, eating, drinking, attempt to escape, defaecation and urination were observed as outlined by Knowles et al. (1999). Observations were done in 30 min interval during the 3-h periods. The number of goat exhibiting each behavioural activity was thus recorded.

Statistical Analysis

The data obtained were expressed as mean ± standard error of the mean (mean ± SEM), and they were subjected to statistical analysis using Student's t-test. Excitability score and behavioural activities for each period of measurement were summed and results are presented as percentiles before subjecting to statistical analysis using Student's t-test. Values of $P < 0.05$ were considered significant.

Results

Table 1: Meteorological Parameters during the Study Period

Meteorological Parameters	
Ambient Temperature Maximum (^{0}C)	30.29 ± 1.47
Ambient Temperature Minimum (^{0}C)	25.8 ± 1.36
Rainfall (mm)	5.75 ± 0.64
Relative Humidity High (%)	86.00 ± 2.45
Relative Humidity Low (%)	74.00 ± 2.30
Sunshine (hr/day)	4.05 ± 0.54
Wind Speed (m/sec)	2.87 ± 0.45

Meteorological Parameters

The maximum AT of 30.29 ± 1.470 C obtained during the study period was not significantly ($P > 0.05$) different from minimum AT of 25.8 ±

1.360 C recorded during the same period. The relative humidity during the period of study was high and the rainfall during the period was also minimal with a value of 5.75 ± 0.64 mm. Sunshine hour/day during this period was 4.05 ± 0.54 and 2.87 ± 0.45 m/sec was recorded for wind speed (Table 1).

Rectal temperature during the Study Period

The rectal temperature of 39.01 ± 0.07 0 C recorded in the experimental goat on day 1 of confinement was not significantly (P > 0.05) different from a value of 39.01 ± 0.07 0 C obtained in the control goat. However, the value of rectal temperature increased (P < 0.05) in the control goat (39.91 ± 0.05) as against 38.73 ± 0.020 C in the experimental goat on the 7th day of confinement. On the 14th day there was a reduction in the rectal temperature in both experimental and control compared to the other days, but however a significant (P < 0.05) decrease was seen in the experimental goats (Figure 1).

Table 2: Haematological Parameters of West African Dwarf Goat Pre-Confinement

Parameters	Experimental	Control
Packed Cell volume (%)	30.29 ± 1.13	31.29 ± 1.30
Haemoglobin Concentration (gm/dl)	10.09 ± 0.38	10.43 ± 0.43
Total Erythrocyte Count (x 10^6µl)	13.46 ± 0.68	16.60 ± 1.82
Total Leukocyte Count (x 10^3µl)	7.57 ± 0.64	7.80 ± 1.33

Haematological Parameters Pre-Confinement and Post-Confinement

The PCV of 30.29 ± 1.13 % obtained in the experimental animal pre-confinement was not significantly (P < 0.05) different from the value of 31.29 ± 1.30 % obtained in the control animal, however other haematological parameters; haemoglobin concentration, total erythrocyte count and total leukocyte count were not significantly (P > 0.05) different (Table 2). The value of leukocyte (7.57 ± 0.37 x 103/µl) recorded in the experimental animal post-confinement

Table 3: Haematological Parameters of West African Dwarf Goat Post-Confinement

Parameters	Experimental	Control
Packed Cell volume (%)	27.86 ± 1.37^a	27.57 ± 1.21^b
Haemoglobin Concentration (gm/dl)	9.19 ± 1.28^a	9.14 ± 0.40^b
Total Erythrocyte Count (x 10^6µl)	15.46 ± 1.02^a	14.49 ± 0.58^b
Total Leukocyte Count (x 10^3µl)	7.57 ± 0.37^a	9.23 ± 0.85^b

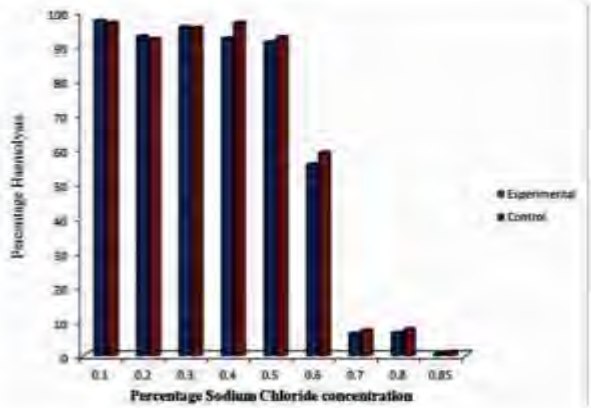

Figure 2: Erythrocyte Osmotic Fragility of West African Dwarf Goat Pre-Confinement

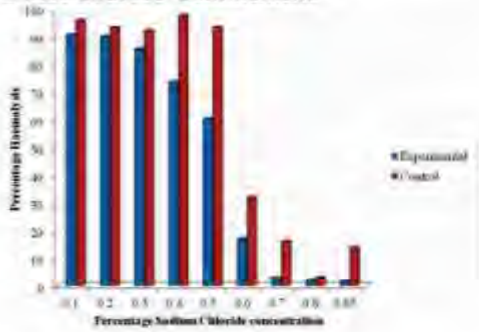

Figure 3: Erythrocyte Osmotic Fragility of West African Dwarf Goat Post-Confinement

was significantly ($P < 0.05$) lower than 9.23 ± 0.85 x 103/µl obtained in the control animals while the obtained values of PCV (27.86 ± 1.37 %), haemoglobin concentration (9.19 ± 1.28 gm %) and total erythrocyte count (15.46 ± 1.02 x 106/µl) obtained in experimental animals was significantly ($P < 0.05$) higher than the corresponding values of 27.57 ± 1.21 %, 9.14 ± 0.40 gm % and 14.49 ± 0.58 x 106/µl respectively in control animals (Table 3).

Erythrocyte Osmotic Fragility

The lowest percentage haemolysis of 0.53 ± 0.23 % was recorded in experimental animals at percentage sodium chloride concentration of 0.85 which was not significantly ($P > 0.05$) different from a value of 1.1 ± 0.18 % in the control animals. Also at 0.5 percentage sodium chloride concentration with a value of 92.53 ± 1.67 % haemolysis was obtained in the control animal which was not significantly ($P > 0.05$) different from the corresponding value of 91.03 ± 4.26 %. However there was no significant ($P > 0.05$) difference between experimental and control animals at any other concentration pre-confinement (Figure 2).

Post confinement the highest value of 95.94 ± 5.66 % was obtained in the control animal at 0.1 % of sodium chloride concentration while the lowest haemolysis of 1.58 ± 0.62 % was obtained in the experimental animal at 0.85 % sodium chloride. The value of 92.16 ± 5.58 % obtained in control animal was significantly ($P < 0.05$) higher than 85.57 ± 5.35 % obtained at 0.3 % sodium chloride concentration. Also the value of percentage haemolysis obtained at 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.85 where significantly ($P < 0.05$) higher in control animal than in experimental animal (Figure 3).

Behavioural Activities in Confined Goats

All the observed behavioural activities pre-confinement was not significantly ($P > 0.05$) different in both experimental and control goats (Figure 4). During confinement the behavioural activities of standing (71.43 %) in experimental animals was significant ($P > 0.05$) higher than the value of 42.86 % in control animals. Quite also the behavioural activities of eating and drinking with a value

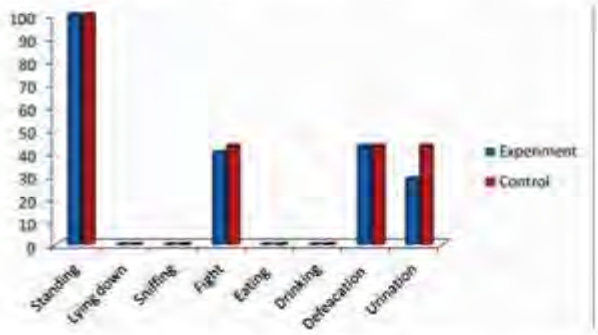

Figure 4: Behavioural Activities of West African Dwarf Goat Pre-Confinement

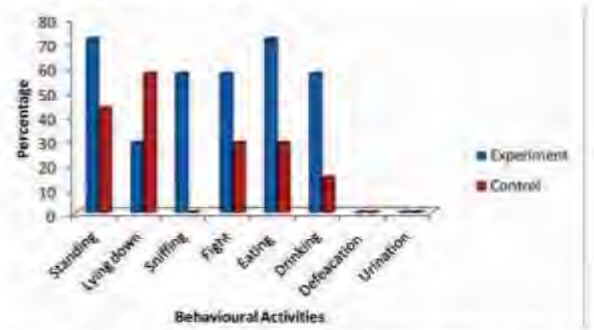

Figure 5: Behavioural Activities of West African Dwarf Goat duing Confinement

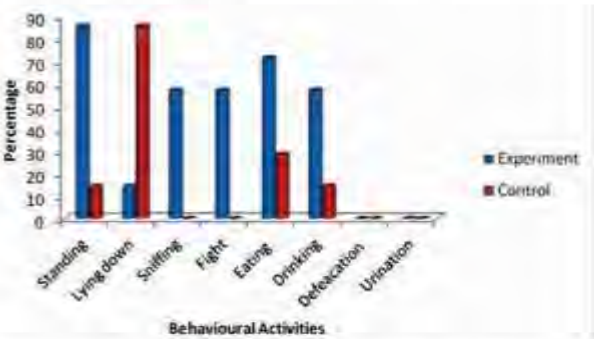

Figure 6: Behavioural Activities of West African Dwarf Goat Post-Confinement

of 71.43 % and 57.14 % respectively was significantly ($P < 0.05$) greater in experimental animals than the corresponding value of 28.57 % and 14.29 % obtained in the control animal respectively while a value of 57.14 % recorded for behavioural activities of lying down in

control animals was greater than 28.57 % recorded in experimental animals during confinement (Figure 5). Post-confinement (Figure 6) the behavioural activities of standing (85.71 %), sniffing (57.14 %), fighting (57.14 %), eating (71.43 %) and drinking (57.14 %) was significantly ($P < 0.05$) higher in experimental animals compared to the corresponding values in the control animals.

Excitability score

During pre-confinement a value of 14.29 % obtained in score 1 in experimental animal was not significantly different from a score of 28.57 % obtained in control animals (Figure 7). During confinement a score of 42.86 % was obtained in experimental animals which were significantly greater than the value of 0 obtained in experimental animal while a score of 2, 3 and 4 with a value of 57.14 % and 42.86 % was significantly higher in experimental animals (Figure 8). Post-confinement a value of 14.29 % obtained in score one and 85.71 % obtained in score 2 in experimental animals was significantly higher than the corresponding value of 0 and 14.29 % obtained in experimental animals while 71.43 % was obtained in score 4 in experimental animals which was significantly greater than that obtained in control animals (Figure 9).

Body Weight

A value of 9.57 kg obtained in experimental animals was not significantly ($P > 0.05$) different from a value of 9.43 kg recorded in control animals pre-confinement. However the weight of animal post-confinement in experimental animals (14.00 kg) was significantly higher than 9.4 kg recorded in control animals.

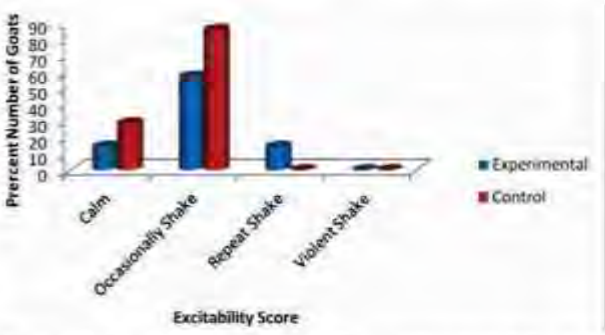

Figure 7: Excitability Scores of Exprimental and Control West African Dwarf Goat Pre-Confinement

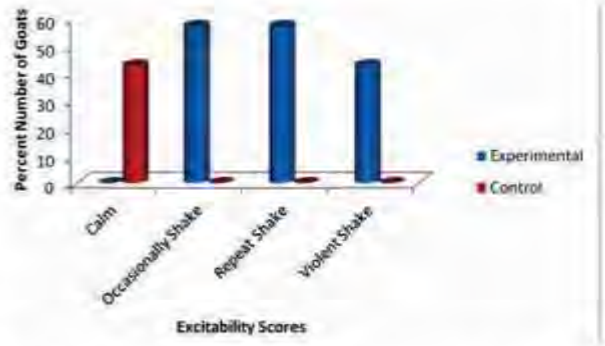

Figure 8: Excitability Scores of Exprimental and Control West African Dwarf Goat during Confinement

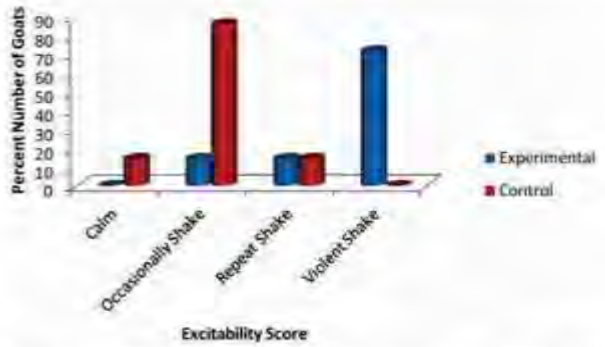

Figure 9: Excitability Scores of Exprimental and Control West African Dwarf Goat Post- Confinement

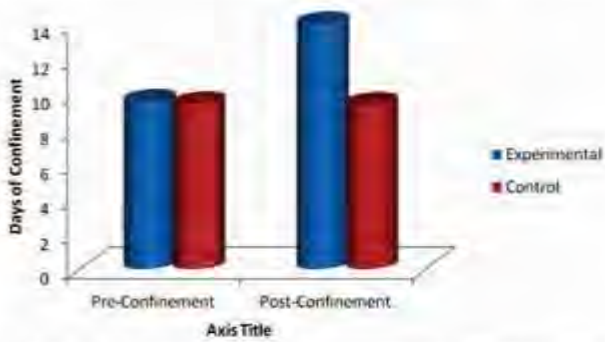

Figure 10: Body weight of Exprimental and Control West African Dwarf Goat Pre and Post- Confinement

DISCUSSION

Meteorological Data during Confinement Period

The obtained maximum and minimum AT during the study period was not significantly different as against what was recorded by Adenkola et al. (2009) during harmattan season in which existed a wide range fluctuations in AT and relative humidity which adversely affects a variety of physiological responses. The reported finding during the harmattan season by these authors was also in contradiction with the meteorological report obtained by Adenkola and Agbendeh (2010) in the same locality during the hot dry season. Rainy season has been described as the least stressful season of all the three seasons (harmattan, hot-dry and rainy seasons) in Nigeria (Igono et al., 1982; Oladele et al., 2003). The meteorological parameters obtained during the study period were within the established thermo neutral zone of 250 C – 300 C for ruminant (Tarr, 2007) in the tropics and this condition did not have adverse effect on their homeostatic mechanism.

Rectal Temperature during Confinement

It has been established that measuring RT which is a true reflection of internal body temperature and a reliable index of thermal balance could be used in evaluating heat stress in livestock (Adenkola et al., 2009). Thus physiologic responses of animals exposed to meteorological stress are determined by verifications in RT (Gordons, 2009). The recorded RT on the first day of confinement for experimental was not significantly different from that obtained in control, this probably could be due to fact that the effect of ascorbic acid has not been felt in experimental animal as RT was measured immediately after AA administration. This finding agreed with the results of the pharmacokinetic study on AA, that AA exerts its effects following 30 min of its oral administration in humans (Hickey et al., 2008). However on day 7 and 14 of confinement, it was observed that the rectal temperature in experimental animals was lower than the control animals and even lower than that obtained in experimental animals on day one of confinement. This finding demonstrated that AA ameliorated the adverse effect of stress by modulating and reducing the RT values in experimental goats. Although the mechanism of AA in reducing RT was not investigated in this study, but however Whitehead and Keller, (2003) postulated that the hypothermic effect of AA could possibly be due to the fact that AA potentiating gamma amino butyric acid (GABA), a powerful inhibitory neurotransmitter in the brain. This finding agrees with the earlier work of Tauler et al. (2003) and Adenkola et al. (2009) that AA supplementation resulted in the maintenance of RT values in livestock. Furthermore, Karanth et al. (2000, 2004) showed that AA is a vitaminergic neurotransmitter released from the hypothalamus and involved in thermoregulation, and that the release was mediated by nitric oxide. The findings in this study suggested that AA exerts both peripheral and central effects on the amelioration of adverse effects of animals subjected to stress situations, this however requires further investigation.

Excitability score

The result of this study pre-confinement showed that the excitability score (ES) which is a function of higher center activities were the same in both experimental and control animals and

most of the animals seemed not to be excited but however during confinement the ES increased more in experimental animals that was administered with AA. This finding is in agreement with that of Ayo et al., 2006, Asala et al. (2010) who demonstrated that AA increased ES in animals under stressful situation and this further confirmed the fact that AA protects the cholinergic receptors from free radical induced damage (Venkatesham et al., 2005). These

finding is also in agreement with the result obtained by Chakraborti et al., 2008 which showed the pre- treatement with antioxidant consistently reversed stress-induced neuro-behavioural changes in rats, and that environmental stress factors have been shown to cause oxidative stress and impair activities of antioxidant in vivo (Halliwell and Gutteridge, 1989, Sahin et al., 2001). The study thus demonstrated the fact that AA improves brain function and mood as observed by Karanth et al. (2000) as this was evident in experimental animals with higher ES post-confinement.

Body weight

The decrease in live weight seen in the control animals post-confinement demonstrates that confinement management practice in goats was very stressful and has adverse effect on the wellbeing of this animal. Conversely there was an appreciable increase in weight of the experimental animals that was administered with AA. The finding in this study is in agreement with the report of Adenkola and Anugwa, (2007) who demonstrated that AA supplementation enhanced full weight gain and better feed utilization in piglets.

Behavioural activities

The fact that most of the experimental animals still remained standing and majority of the control animals were lying down during confinement is an indication that confinement as a management practice is stressful to this animals while the experimental animals that remained standing indicated that there was a better neuromuscular co-ordination and that AA prevented muscular damage often caused by free radical production leading to muscle glycogen depletion, fatigue and lethargy (Powers and Jackson, 2008). AA is essential for the synthesis of muscle carnitine required for transport and transfer of fatty acid into mitochondria were it can be used for energy. The behavoural activity of sniffing which was higher during confinement in experimental animals could be attributed to the fact that AA stimulates libido in those group of animals. This is in agreement with the findings of Yousef (2005) that AA enhanced libido in animals. Eating and drinking was more in the experimental animals while majority of the control animals went off feed during confinement. This supports the findings of Yen and pond (1981) that AA supplementation improved both daily feed intake and daily weight gain in pigs. AA works as a co-enzyme playing an important role in metabolism of amino acid (Kutler and Forbes, 1993). The fact that many of the experimental animals were involved in fighting could be due to the effect of AA, as it has been found to act as a co-factor for the enzyme dopamine beta hydroxylase, which catalyzes the conversion of the neurotransmitter dopamine to adrenaline (Karanth et al., 2000) and the aggressive effect seen could be due to adrenaline release.

Haematological Parameter

The haematological parameters of PCV, Hb and total RBC count which was higher in the experimental animals in this study still collaborates with the findings of EOF studies in which more haemolysis was seen in the control animal compared to the experimental animals which was administered with AA post-confinement. Free radicals were not measured directly in this study but it has been shown that they are generated in this animals subjected to stress (Senturk et al., 2001, Chihuailaf et al., 2002). An EOF study has been used as an indirect way of measuring free radicals (Adenkola and Ayo, 2009). The membrane of erythrocyte is rich in polyunsaturated fatty acid which is susceptible to lipid peroxidation and resultant loss of membrane fluidity and cellular lysis (Brzezinska Stebodziiska, 2003) which then resulted in higher haemolysis in the control animal which could be brought about as a result of the effect of confinement stress in the RBC. However in the experimental animals the percentage haemolysis was lowered, this could be due to the fact that AA consolidates the

membrane integrity of erythrocyte membrane and therefore reduces their oxidative damage. Oxidative stress occurs when the antioxidant defense system in the body are overwhelmed by free radicals (Williams et al., 2008). The finding in this study agrees with the earlier work of Candan et al. (2002) and that of Adenkola and Ayo (2009) that AA protected the membrane integrity of the RBC.

The observed increase in total leukocyte count in control animals during confinement which was demonstrated to be stressful (Adenkola and Ayo, 2006) and, also the activities of the hypothalamo-pituitary adrenal axis resulting in the release of the glucocorticoids from the adrenal axis. In the experimental animals ascorbic acid may have likely prevented the release of leukocytes from their pool in the body into peripheral circulation, apparently due to its role in circulating glucocorticoids in animals under stress. A decrease in AA body tissue especially in adrenal glands is known to be associated with corticosteroid release (Whitehead and Keller, 2003).

In conclusion confinement as a management practice during the rainy season constitutes stress which could adversely affect the health and productivity of goats as indicated by the parameters determined, but this adverse effect could be modulated by administration of non-toxic, non-expensive, readily available antioxidant agent like AA.

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