Alexandria Journal of Veterinary Sciences
www.alexjvs.com

AJVS. Vol. 66 (1): 10-14 Jul 2020
DOI: 10.5455/ajvs.94532

Erythrocyte Osmotic Fragility of Pubertal Red Sokoto Goats Administered Ascorbic Acid During the Early Rainy Season

Taiwo K. Bello[1*], Joseph O. Ayo[2], Balkisu B. Oyelowo[3]
[1]National Animal Production Research Institute, Shika-Zaria, [2]Department of Veterinary Physiology, Ahmadu Bello University, Samaru-Zaria, [3]Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Samaru-Zaria

Key words:
Red Sokoto Goat, Ascorbic Acid, Erythrocyte Osmotic Fragility

***Correspondence to:** :
taiwokamarbello@gmail.com,

Article History
Received: 22 Mar 2020
Accepted: 25 May 2020

ABSTRACT

Experiments were performed with the aim of determining the effect of administration of ascorbic acid (AA) on erythrocyte osmotic fragility of pubertal Red Sokoto goats during the early raining season. Ten goats of both sexes served as subjects for the study. Five goats administered orally with AA at a dose rate of 100 mg/kg served as experimental animals, while five goats administered orally with distilled water served as controls. Blood samples were collected a day before AA administration and on days 3 and 7 post-administration of AA to determine erythrocyte osmotic fragility in the goats. A significant ($P < 0.05$) decrease in percentage of erythrocyte osmotic fragility was recorded on days 3 and 7 post-administration of AA. The results showed that AA ameliorated environmental stress by reducing the percentage of erythrocyte osmotic fragility in the goats.

1. INTRODUCTION

Stress is a consequence of adverse effects of environment or management systems, which force changes in an animal's physiology or behaviour to avoid physiological malfunctioning. It therefore, assists the animal to cope with its environment. Stress is commonly used to describe the detrimental effects of environmental conditions on the health and performance of animals. Environmental stress causes an increase in oxidative stress, resulting in an imbalance in antioxidant status (Ahmadu et al., 2016). In heat stress, free radicals are generated in the body in such large quantity that the natural antioxidant defence systems of the body are overwhelmed (Sahota and Gillani, 1995; Altan et al., 2003). This results in increased lipid peroxidation of cytomembranes; and consequently, cell damage and destruction (Freeman and Crapo, 1982). Adverse states in animals are assessed based on changes in physiological, including haemtological and biochemical parameters (Fazio et al., 2003). Modern livestock production is a highly specialised industry, which exposes food animals to different stressful conditions. In tropical countries, including the West African sub-region, heat stress is more common, especially during the hot-dry season (Ayo et al., 1998).

Dietary management of stress includes the use of antioxidants and it has great potential application in the amelioration of stressful conditions in livestock production.

The Northern Guinea Savannah zone of Nigeria is characterized by wide ambient temperature variations due to high solar radiation intensity combined with a longer day. Due to the scourge of global warming and climate change, goats are also vulnerable to various kinds of stressors, especially thermal stress in tropical countries. As climate change is further expected to intensify in the future, the susceptibility of small ruminants to heat stress is also expected to aggravate, thereby compromising the welfare of animals (Reddy et al., 2019). Reduced feed intake occurs in animals exposed to hot environment, and this explains the biological mechanism by which climatic stressors impacts production and reproduction. This includes reduction in rumination and nutrient absorption, altered endocrine status, and increased maintenance requirements resulting in a net decrease in nutrient/energy availability (Conte et al., 2018).

Ascorbic Acid (AA) is a water-soluble antioxidant vitamin, non-toxic, sustainable and readily metabolised by the body of most domestic animals and humans (Padayatty et al., 2003; Hickey et al., 2008). AA is a carbohydrate-like substance involved in metabolic functions, including synthesis of collagen, maintenance of the structure, strength of blood vessels or release of hormones in the adrenal glands (Halliwell, 2012). When administered orally, its absorption takes about 30 minutes (Pardue et al., 1984; Hickey et al., 2008). Ascorbic acid has potent antioxidant properties; that is, it is able to reduce damage caused by reactive oxygen species (ROS). Ascorbic acid is a water-soluble chain breaking antioxidant. It scavenges free radicals and ROS (Yimcharoen et al., 2019). In order to scavenge the increased generation of ROS, which can alter biologically cellular macromolecules and can interfere with cell signaling pathways (Akbarian et al., 2016), a wide variety of antioxidants has been evolved (Halliwell, 2012). Ascorbic acid (AA) reduces ROS- induced damage by directly binding to ROS converting them to less harmful molecules. Erythrocytes are susceptible to oxidative stress as a result of high contents of unsaturated fatty acids in their membranes (Altan et al., 2003). Erythrocyte osmotic fragility (EOF) test is a measure of erythrocyte strength and its ability to withstand varying osmotic gradients (Aldrich et al., 2006). Osmotic stability and fragility of erythrocytes are related measurable quantities in haematology which estimate haemolysis under hypoosmotic stress (Igbokwe et al., 2018). It is related to the geometrical configuration of the erythrocyte. In hypotonic environment, the erythrocyte swells to its maximum, critical volume, and becomes spherical before being haemolysed. Further increase in cell volume by continuous hypotonic stress stretches the erythrocyte membrane, leading to the formation of pores that are large enough to permit leakage of haemoglobin. The EOF test is used to assess the stability of erythrocytes in hypotonic solutions (Oladele et al., 2003; Asala et al., 2011). Reddy et al., (2019) hypothesize that EOF could be specifically used to assess the welfare compromise and resilience in animals over a period of prolonged stress. Changes in temperature and pH of the erythrocyte environment and duration of blood storage may each play a significant role in the osmotic behaviour of RBC (Oyewale et al., 2011). The effect of temperature has been demonstrated to be exerted on the membrane lipids and proteins of the erythrocytes (Oyewale et al., 1997).

The high environmental temperature decreases the concentrations of vitamins and micro minerals in serum and increase the excretion. Therefore, supplementation with direct or indirect antioxidant compounds like AA is commonly recommended (Horváth and Babinszky, 2019). Heat stress impairs absorption of AA and, thus, increases the body requirements in the vitamin (Naziroglu et al., 2000). When the concentration of antioxidant vitamin decreases, lipid peroxidation increases in the plasma and tissues, leading to cell membrane damage (Sahin et al., 2002). The Northern Guinea Savannah zone has two major seasons - dry and rainy seasons, lasting for 5-6 months and for 6-7 months respectively. The cold-dry (harmattan) period stretches from November to February and sometimes March, while the hot-dry period is from March to April/May. As the rainy season sets in, the weather becomes very hot and humid. The period with the greatest heat stress for animals in the zone, therefore, covers March to May (Balogun et al., 1993).

There is paucity of information no studies in the available literature on the effect of stress due to hot and humid environment during the early raining season on EOF of Red Sokoto goats, and measures to mitigate the stress. Such information if available, may improve the welfare, ameliorate stress and increase productivity and profitability.

The aim of the present study was to evaluate the response of erythrocytes of goats to osmotic stress and the effect of ascorbic acid in goats under hot-humid conditions.

2. MATERIALS AND METHODS

Ten healthy pubertal Savannah Brown goats of both sexes approximately 2-3 years' old and weighing 20-25 kg served as subjects. The goats were kept in a pen made of concrete floor, cement block wall and wire-mesh with asbestos roofing. The pens measured 2.42 m × 7.39 m wide and 1.12 m high and of wire-mesh from the floor, which provided adequate ventilation. The goats were kept under a semi-intensive system of management. They were allowed to graze during the day and fed with beans offal later in the evening. They were given access to water *ad libitum*. This study was conducted in the month of May, 2013.

On experimental day, ascorbic acid was administered *per os* to five experimental goats at 08:00 h at the dose of 100 mg/kg (Chervyakov et al., 1977), dissolved in 10 ml of sterile water. Exactly 10 ml of sterile water was administered *per os* to five goats that served as controls. At the same hourly period, the dry- and wet-bulb thermometer readings were taken inside the animal pen

Table 1: Mean temperature humidity index during the study period

Hour	Temperature Humidity Index
07.00	70.72
08.00	74.92
09.00	80.84
10.00	81.20
11.00	82.48
12.00	82.40
13.00	82.40
14.00	83.68
15.00	81.32
16.00	81.04
17.00	80.68
18.00	80.40
19.00	78.40
Overall mean ± SEM	80.1 ± 1.6

Before ascorbic acid administration, 3 ml of blood sample was drawn aseptically from each goat by jugular venipuncture, using a 10-ml syringe and 21-gauge sterile needle. The blood was immediately poured into sample bottles, one for each goat, containing the anticoagulant heparin at 20 IU/ml of the blood sample. After collection, the samples were transferred to Physiology Research Laboratory, Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria to determine the EOF. Blood was collected from each animal on days 3 and 7 after administration of ascorbic acid. The samples were transferred to Physiology Research Laboratory, Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria to determine the EOF. The data were expressed as means ± standard error of the mean (Mean ± SEM). Student's t-test was used to determine significant differences between the control and experimental goats. Values of $P < 0.05$ were considered significant.

3. RESULTS

The mean temperature-humidity index was 80.1 ± 1.6. Percentage haemolysis on day 3 post-administration of ascorbic acid had a maximum value of 80.0 ± 9.1% at 0.1% NaCl concentration in experimental goats (Fig. 2), while in control goats, the maximum value of 96.0 ± 4% was recorded at 0.9% NaCl concentration (Fig. 1). On day 7 post-administration of ascorbic acid, haemolysis was still highest at 0.1% NaCl concentration with a value of 93.5 ± 6.5% (Fig. 2). In the control goats, the highest recorded haemolysis was recorded at 0.1% NaCl concentration on both days 3 and 7 post-administration, with values of 96.4 ± 4% and 96.2 ± 1.8%, respectively (Fig. 1).

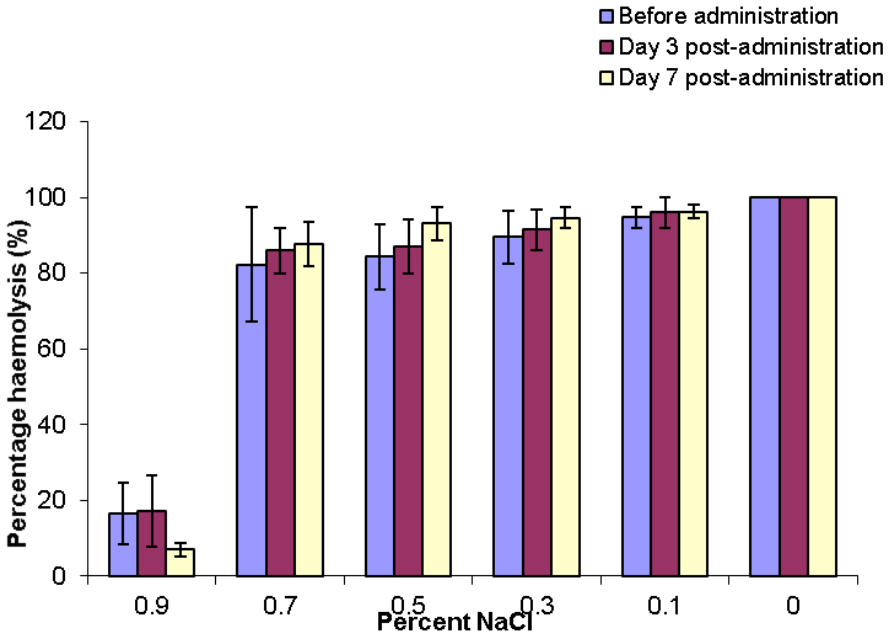

Fig. 1: Osmotic Fragility in Control Red Sokoto Goats (n = 5) not administered with Ascorbic Acid

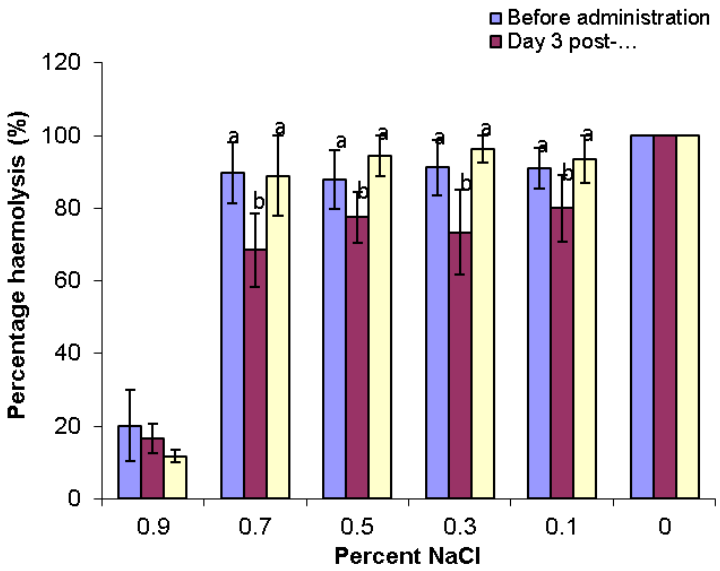

Fig. 2: Osmotic Fragility of Erythrocytes in Experimental Goats (n = 5) administered with Ascorbic Acid. Bars with different superscript differ significantly (P< 0.05).

4. DISCUSSION

The values obtained during the study period showed that the early rainy season was characterised by high relative humidity index values. Thus, the hot-humid period was a stressful condition for goats (Serradilla et al., 2015). The results of the EOF showed that there was a decreased haemolysis on day 3 post-administration of ascorbic acid in experimental goats. This may be attributed to the effect of ascorbic on free radicals, which protected tissues and cell membranes; thus, reducing the fragility of erythrocyte membranes and consequently, haemolysis. However, on day 7 post-administration of ascorbic acid, there was an increase in haemolysis in the experimental goats as compared to values obtained in day 3. The result may be attributed to the depletion of the administered ascorbic acid from the body system, indicating that ascorbic acid exerted a transient decrease in haemolysis following its administration. It has been shown that erythrocytes are highly susceptible to oxidative stress as a result of high amount of unsaturated fatty acid contents in their membranes (Atlan et al., 2003). This further supports the finding that increased ROS generation occurs in stress, decrease antioxidants in the body (Tauler et al., 2003; Nazifi et al., 2009). In contrast in the control goats, the percentage haemolysis was consistently high through day 0 to 7. This finding may be attributed to the absence or low level of antioxidants, including ascorbic acid, involved in ameliorating the deleterious effects of ROS on the erythrocyte membranes. The high percentage result of EOF obtained in this study demonstrated an increased erythrocyte membrane fragility in the control goats, apparently due to ROS induced modulation of the molecular properties of the erythrocyte membrane (Ayo et al., 2015).

The EOF percentages recorded in goats administered with ascorbic acid were significantly lower (P > 0.05) on day 3 of the study period, suggesting that ascorbic acid exerted protective effect on the erythrocytes from haemolysis in goats during the early raining season. The finding also suggests that administration of ascorbic acid to goats during the early raining season ameliorated the negative effects of the hot-humid climatic conditions. Ascorbic acid has been demonstrated to reduce the capacity of O_2 consumption through tissue oxidative metabolism and decrease heat load by enhancing mechanism of thermoregulation via increasing heat loss (Kuth and Forbes 1993), which may also decrease ROS generation of free radicals and further reduce lipoperoxidation (Ayo et al., 2015).

5. CONCLUSION

The administration of ascorbic acid protected and stabilised erythrocyte membrane integrity by reducing considerably the percentage haemolysis on day 3 post-administration of ascorbic acid. The administration of ascorbic acid for 3 days may be beneficial to goats during the early rainy season.

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