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Physiological Responses of Erythrocytes of Goats to Transportation and the Mondulatory Role of Ascorbic Acid

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ABSTRACT. Experiments were performed with the aim of investigating the effect of road transportation for 12 hr on erythrocytes of goats during the hot-dry season and the modulatory role of ascorbic acid. Forty 2.5–3-year-old Red Sokoto goats weighing 23–25 kg and belonging to both sexes served as the subjects of the study. Twenty of the goats served as the experimental group and were administered ascorbic acid (AA) *per os* at a dosage rate of 100 mg/kg body weight; the other 20 served as controls and were given 10 m*l* each of sterile water. Forty minutes after the administration and loading, the goats were transported for 12 hr. EDTA blood samples collected before loading, after loading, immediately after transportation and subsequently on the 3rd and 7th days of post-transportation were used to determine the red blood cell (RBC) count, packed cell volume (PCV), hemoglobin (Hb), erythrocyte osmotic fragility (EOF), hematimetric (intrinsic) indices and hemoglobin index levels. The obtained results showed that handling, loading and transportation of the control goats induced significant ($P<0.05$) increases in RBC, Hb, EOF and hypochromic erythrocytes and a decrease ($P<0.05$) in the volume and average Hb content in RBCs. AA administration ameliorated all these changes. The present results suggest that road transportation for 12 hr during the hot-dry season could induce serious stress, resulting in hemolysis of erythrocytes, which was ameliorated by AA administration. In addition, the results demonstrated that EOF could be used as a diagnostic tool in road transportation stress.
KEY WORDS: Ascorbic acid, erythrocytes, goats, hemolysis, transportation stress.

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Road transportation of livestock is a stress-inducing situation that may lead to psychological and physiological insult. Several studies have reported the responses of different physiological parameters to the effect of road transportation stress in other species of livestock [22, 27, 30]. In goats such studies are grossly inadequate [18, 24]. Few reports have shown that goats are highly susceptibility to diseases following prolonged transportation by road under adverse climatic conditions [18]. The major stress factor affecting goats reared in regions with hot-climatic conditions and predominately raised under an extensive management system during road transportation are those of rounding-up, handling and loading and the adverse effect of high ambient temperature and relative humidity during the journey.

Blood examinations are routinely used in diagnosis of the health status and evaluation of adaptability of livestock to stress factors [22, 23]. One of the important blood parameters that may be measured during stress is erythrocytes. This is because erythrocytes are the chief cells that circulate oxygen in an organism and are highly susceptible to oxidative damage induced by free radicals or reactive oxygen species (ROS) generated under stressful conditions [20, 39]. Studies in human subjects [39], rats [35] and horses [29] exposed to exhaustive muscular exercise have shown considerable reduction of natural antioxidants and generation of ROS in the body, resulting in alteration of erythrocyte cytomembranes, and hemolysis. This subject is extensively reviewed by Powers and Jackson [31].

Testing of the osmotic fragility of erythrocytes and its intrinsic parameters provide an indication of the ratio of surface area/volume for erythrocytes [28, 34]. The EOF is used to evaluate the adaptability of the erythrocytes, degree of hemolysis, diagnose hereditary anemia, and for screening in blood banks through the use of a single point bag [14, 34].

There is a paucity of information on the physiological responses of goats erythrocytes to the effect of long distance road transportation stress during the hot-dry season, and measures to alleviate such stresses are lacking in the available literature. Even though the effect of AA on stress-stimulated oxidative damage remains controversial, there is a reasonable body of evidence supporting the use of AA as an anti-stress agent [5, 7, 24, 31].

Although ruminants may not require AA supplementation in the diet, the requirement of AA may exceed the synthetic capacity of the liver during stress, diseases or exercise. Supplementation of this vitamin at the moment of greatest need for the animal can be a potential reserve and an alternative treatment at low cost [23, 24].

The aims of the present study were to investigate the responses of erythrocytes of goats subjected to 12 hr of road transportation during the hot-dry season and to suggest AA, as a measure for alleviating hemolysis.

MATERIALS AND METHODS

Animals and management: Forty Red Sokoto goats, including males and non-pregnant females, two-and-half to three years old and weighing between 23–25 kg served as

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the subjects of this study. They were reared under the traditional extensive management system and were housed at night in a standard goat pen. The goats were not restrained inside the pen and were stocked at a rate of 1 m^2/goat. Each day the goats were herded out and grazed on an improved natural pasture. Three weeks before transportation, the goats were screened for diseases and prophylactically treated against ecto- and endoparasites.

Experimental design: The experiment was conducted at the Livestock Farm of the College of Agriculture and Animal Science, Ahmadu Bello University, Kaduna (11° 10'N, 07° 38'E), located in the Northern Guinea Savannah zone of Nigeria. Transportation of the goats was conducted during the hot-dry season (April) from Kaduna to Makera (12° 31'N, 06° 11'E) and from Makera back to Kaduna. The ambient temperature (AT) and relative humidity (RH) were recorded at the experimental site at 06:00, 13:00 and 18:00 hr daily for seven consecutive days before and after transportation. The dry-bulb temperature and relative humidity were measured using a wet- and dry-bulb thermometer (Cocet, Shenzhen,Guangdong, PR China). The Values of AT and RH were also recorded every hour of the 12-H transportation period and after unloading the goats from the vehicle during the first four hours post-transportation.

One week before transportation blood samples were collected from 20 goats selected at random to determine baseline values. On transportation day, before the journey the goats were randomly allocated into experimental and control goat groups, consisting of 20 goats each. During handling at 06:00 hr before loading, a 5-H m*l* blood sample was collected from ten goats in each group in order to obtain preloading baseline values. The goats from which blood samples were collected were colored-marked for easy identification. Within the same period, all the experimental goats were orally administered AA (Sigma Chemical, St. Louis, MO, U.S.A.) at a dose of 100 mg/kg body weight [5, 24] dissolved in 10 m*l* of sterile water, while each of the control goats was orally administered 10 m*l* of sterile water only. Handling and loading of the goats took about 40 min. After loading and just before commencement of the journey, blood samples were quickly obtained from the other 10 goats of each group that had not been subjected to blood sampling previously to determine the effects of handling and loading. This design was utilized to eliminate the compounding effects of repeated handling and bleeding of the same goats within a short time [25]. After blood sampling, all the goats were transported. Food was withdrawn from the goats 6 hr before transportation and throughout the 12-hr journey period, but water was withdrawn only during the journey period [13, 32].

On completion of the journey, the goats were returned to the same pen, and offered feed and water and were managed as before their transportation. Immediately after transportation, 12 hr after and on the 3rd and 7th days of the post-transportation period, blood samples were collected from all the goats.

Handling, loading and transportation of the goats were carried out humanely in accordance with the guidelines governing animal transport welfare by road [3, 12]. All handling and loading were conducted between 06:00 to 07:00 am.

A standard Bedford pick-up van (Made in England) with no top roof was used for the journey. The floor of the vehicle was provided with wood shavings as the beddings and was covered with rubber mats for secured footing. The goats were stocked at a rate of 0.3 m^2 per animal. The vehicle travelled for 12 hr (07:00–19:00 hr) on a typical asphalt road, covering a total distance of about 600 km, with an average speed of 50 km/hr. During the journey, the vehicle had four stop-overs at veterinary and police control posts, and each lasted less than 5 min. The goats were rested for an hr after 8 hr of transportation period in adherence to international guidelines for transport welfare [3]. During the resting period, the vehicle was parked in the shade to avoid the direct effect of sunlight on the goats and rapid build-up of heat inside the vehicle [32].

Blood sampling and analyses: At each period of blood sampling, 5 milliliters of blood was collected by venipuncture of the jugular vein of each goat using disposable syringes and an 0.8 × 25H-mm needle into a sterile test tube with anticoagulant (EDTA K3, Pty Ltd., Adelaide, SA, Australia). The needles were removed to prevent mechanical hemolysis when the blood was being transferred to the collection tubes. The collected blood samples were analyzed for red blood cells (RBC), hemoglobin (Hb) and packed cell volume (PCV) as described by Schalm *et al*. [33]. The following hematimetric (intrinsic) indices were calculated: mean corpuscular volume (MCV: PCV × 10/RBC), mean corpuscular hemoglobin concentration (MCHC: Hb × 100/PCV), mean corpuscular hemoglobin (MCH: Hb × 10/RBC) and hemoglobin index (HI) (obtained Hb × average RBC/average Hb × obtained RBC) [33].

Erythrocyte osmotic fragility (EOF) was determined as described by Beutler [8] and Oyewale [28]. Hemolysis in each tube was expressed as a percentage, taking 100% as the maximum value of the absorbance of the distilled water (0% concentration).

Statistical analysis: The data are expressed as means ± standard error of the mean (Mean ± SE). The Student's *t*-test was used to determine significant differences between the control and experimental goats. ANOVA and Tukey's test were used to analyze the effects of the sampling periods (loading and post-transportation periods). Values of $P<0.05$ were considered significant.

RESULTS

Meteorological conditions: The AT measured in the study area had minimum and maximum values of 20.0°C and 39.4°C, respectively, and were obtained correspondingly at 07:00 and 14:00 hr. The AT had a mean value of 36.7 ± 0.2°C, while the RH fluctuated between 65% and 75% with a mean value of 65.5 ± 5.6%. During the transportation especially in the hot-afternoon period of the day,

the AT inside the vehicle rose with the duration of the journey from 28.2°C to a maximum value of 40.1°C at 14:00 hours. Thereafter, the AT decreased to 34.0°C during the last hour of the journey. The mean AT recorded during the journey was 37.2 ± 1.3°C, while the mean RH was 75.4 ± 1.8%.

Red blood cell count and hemoglobin concentration: Table 1 depicts the effects of loading, twelve hours of road transportation and the administration of AA on RBC, Hb, PCV and the erythrocyte intrinsic parameters of the goats. The RBC count and Hb concentration increased post-loading, especially in the control goats. Immediately after transportation and three days later, the RBC and Hb values recorded in the experimental goats were not different from the post-loading values but were significantly (P<0.05) lower than the corresponding values obtained in the control goats. The RBC count in the control goats returned to the pre-loading values seven days after transportation. The PCV showed a significant (P<0.05) increase after loading and transportation in both the experimental and control goats when compared with the pre-loading values. The values returned to baseline levels at 12 hr and 3 days post-transportation in the experimental and control goats, respectively.

The MCV, MCH and MCHC intrinsic parameters were all affected by transportation and AA administration. Loading and transportation decreased the volume (MCV) and Hb (MCH) content in regard to individual RBCs, while MCHC significantly (P<0.05) increased post-loading and during transportation, especially in the control goats.

Erythrocyte osmotic fragility: The erythrocyte osmotic fragility of the goats during pre-loading is shown in Fig. 1a. Fifty percent (median corpuscular fragility) of the erythrocytes were hemolyzed at an NaCl concentration of 0.60% in the control group, and the highest percent hemolysis, 82 ± 4.5%, was recorded at an NaCl concentration of 0.30% in the transported goats and there were no differences between them.

At post-loading and post-transportation, 50% of the erythrocytes were hemolyzed at an NaCl concentration of 0.8% in the control goats, and this value was higher (P<0.05) than the preloading value of 0.6% and also higher than the corresponding values of 0.5–0.7% obtained in the experimental goats (Fig. 1a-e). Similarly, the maximum hemolysis was recorded at an NaCl concentration of 0.5% in the control goats during the post-loading and post-transportation periods, and this value was higher (P<0.05) than the pre-loading value and the corresponding value of 0.3% recorded in the experimental goats during the post-loading period (Fig. 1a-e). Overall, the percent erythrocytes that undergo 50% and maximum hemolysis in the control goats at various NaCl concentrations post-loading were higher than the preloading values and the corresponding values recorded in the erythrocytes of the experimental goats. The values returned to baseline levels at 12 hr after transportation in the experimental goats (Fig. 1d), while in the control

Table 1. Effects of loading, twelve hours of road transportation and administration of ascorbic acid on erythrocyte counts and intrinsic parameters in the experimental and control goats

	Pre-transportation period			Post-transportation period (n=20)			
Parameters	*One week before (n=20)	Pre-load (n=10)	Post-load (n=10)	Immediately	Twelve hours	Three days	Seven days
Erythrocyte (×10⁶/ul):							
Experimental	11.0 ± 0.2	10.0 ± 1.2	11.2 ± 1.0	16.5 ± 0.5^{xy}	12.5 ± 1.4^{z}	11.0 ± 2.4	10.4 ± 1.2
Control	10.8 ± 0.7	9.8 ± 0.5	15.5 ± 0.8^{bx}	24.8 ± 0.7^{bxy}	24.0 ± 1.2^{bx}	22.4 ± 4.2^{bx}	9.4 ± 2.2^{f}
Haemoglobin (g/l):							
Experimental	6.9 ± 0.4	7.3 ± 0.4	8.0 ± 0.4	9.7 ± 0.6^{x}	10.2 ± 0.1	8.6 ± 0.2	7.8 ± 0.8
Control	7.2 ± 0.4	7.4 ± 0.8	9.9 ± 0.3^{x}	16.3 ± 0.7^{bxy}	15.4 ± 0.5^{bx}	12.8 ± 0.8^{bxk}	8.0 ± 0.4^{f}
PCV (%):							
Experimental	21.0 ± 1.4	22.0 ± 1.2	24.7 ± 1.0	29.2 ± 1.0^{x}	24.5 ± 1.4	20.5 ± 1.5	21.5 ± 1.2
Control	20.2 ± 1.6	21.2 ± 1.4	26.1 ± 1.7	29.5 ± 1.2^{x}	26.2 ± 1.0	20.1 ± 1.5	20.5 ± 1.0
MCV (fl):							
Experimental	22.0 ± 1.7	22.0 ± 2.1	22.1 ± 2.2	17.7 ± 1.3^{xy}	19.6 ± 2.2	18.6 ± 3.5	20.7 ± 1.1
Control	20.6 ± 2.1	21.6 ± 1.1	16.8 ± 0.1^{bx}	12.3 ± 2.3^{bxy}	10.9 ± 3.0^{b}	9.1 ± 2.04^{b}	21.8 ± 2.1
MCH (pg):							
Experimental	6.8 ± 0.8	7.5 ± 1.0	7.7 ± 0.8	6.6 ± 0.5	8.2 ± 0.3	7.8 ± 1.2	7.5 ± 2.0
Control	7.2 ± 0.5	7.6 ± 0.5	6.4 ± 0.2^{b}	6.0 ± 0.8	6.4 ± 1.1^{b}	5.8 ± 1.0^{b}	8.5 ± 3.2
MCHC (g/dl):							
Experimental	32.2 ± 4.5	33.2 ± 7.2	34.4 ± 5.5	33.2 ± 3.0	41.6 ± 2.5	42.0 ± 4.5	36.3 ± 4.2
Control	34.4 ± 1.6	34.9 ± 2.6	37.9 ± 4.3	55.3 ± 1.8^{bxy}	58.8 ± 4.5^{bxz}	63.7 ± 2.8^{bxk}	39.0 ± 2.8^{f}
Hb index:							
Experimental	0.90 ± 0.02	0.87 ± 0.04	0.90 ± 0.01	0.98 ± 0.01	0.98 ± 0.02	0.94 ± 0.03	0.90 ± 0.01
Control	0.89 ± 0.03	0.91 ± 0.01	0.76 ± 0.01^{bx}	0.70 ± 0.03^{bx}	0.70 ± 0.01^{bx}	0.68 ± 0.02^{bx}	0.94 ± 0.01^{f}

x=P<0.05 vs pre-load; y=P<0.05 vs post-load; z=P<0.05 vs immediately post-transportation; k=P<0.05 vs 12 hr post-transportation; f=P<0.05 vs three days post-transportation; b=P<0.05 vs experimental. * Baseline values one week before transportation were not included in the statistical analysis.

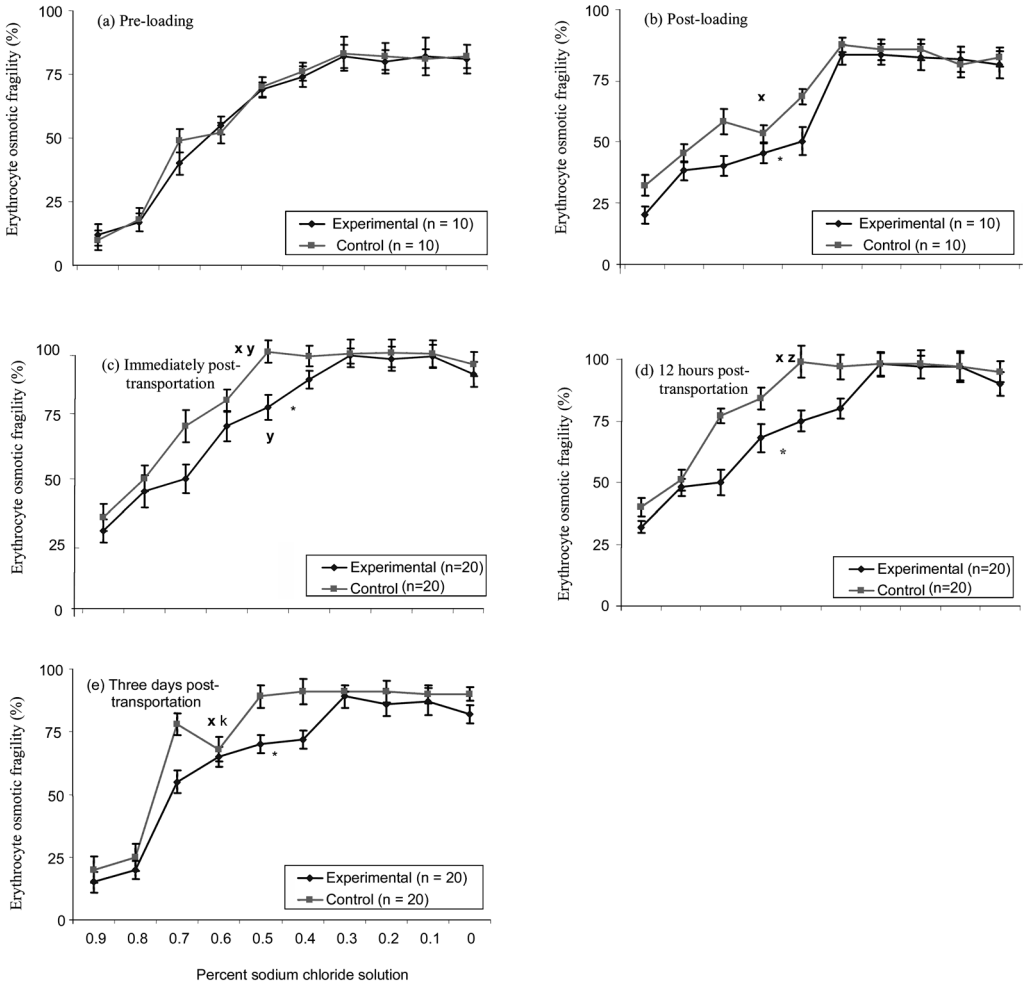

Fig. 1a-e.The erythrocyte osmotic fragility of the experimental and control goats during the pre-loading (a), post-loading (b), immediately post-transportation (c), 12 hr post-transportation (d) and three days post-transportation (e) periods, respectively, at a pH value of 7.4 and temperature of 26°C. Each point represents the mean ± SEM. *=P<0.05 vs control; x = P<0.05 vs pre-loading; y = P<0.05 vs post-loading; z = P<0.05 vs immediately post-transportation; k = P<0.05 vs 12 hr post-transportation.

goats, the values returned to baseline levels at 7 days after transportation (not shown in Figure).

DISCUSSION

The results for the RBC counts, Hb and EOF of the goats obtained one week before transportation and during pre-loading were similar and within the normal ranges of values recorded in tropical goats [17, 28], which suggested that the goats were healthy. The increased RBCs, Hb and EOF recorded post-loading, especially in the control goats, showed that the increase was due to the procedures of rounding-up, handling and loading of the goats, which induced excitement, resulting in splenic contraction and the release of RBCs into the circulation [4, 26, 38, 39]. This mechanism is induced by the action of catecholamines on α-adrenergic receptors located in the splenic capsule and partly attributed to a reduction in the plasma volume [26, 39]. A similar increase in Hb was recorded during handling and isolation of goats by Al-Qarawi and Ali [1]. Handling and loading have been reported to be the most stressful period and to be associated with large increases in the plasma concentrations of adrenocortical (cortisol) and pituitary (prolactin) hormones within the first 10–30 min of handling [1, 4, 10, 22, 30]. The release of these hormones into the blood at an earlier stage of stress is a defense mechanism [39]. However, as the stress factors overcome the negative feedback regulation of the hypothalamus pituitary axis (HPA), a rise in the production of these hormones in turn inhibits the functions of the immune system. In addition, physical and emotional stress as obtained during handling and loading in the present study are known to induce

mechanical damage of erythrocytes and to enhance the mechanism of free radical production, which destroys erythrocytes [2, 34–36, 39]. The insignificant (P>0.05) increase in the RBC count, Hb and EOF of the goats administered AA post-loading suggested for the first time that AA administration may reduce or abolish the adverse effects of handling and loading stress on the erythrocytes of goats before commencement of transportation. The mechanism of action of AA in alleviating the stresses induced by handling and loading is apparently via the potentiation of α-aminobutyric acid (GABA) [9], which in turn inhibits the release of the cortisol and prolactin involved in splenic contraction and cell damage, and probably by increasing the level of plasma and the membrane cholesterol concentration, which protects erythrocytes from peroxidant stress [21, 31, 39, 40]. It has been reported that cholesterol acts as an oxidant radical scavenger by protecting adjacent cytomembrane lipids from oxidative attacks. This oxidative attack begins in areas of erythrocyte membranes with less cholesterol concentration [11, 16, 40]. This means that AA absorbed into the plasma within 20–30 min of its oral administration before transportation may increase the plasma cholesterol level at an earlier state. This may require further investigations. The results obtained from the present study have shown that AA enhanced the antioxidant defense system of the RBC and provided protection from the harmful effects of handling and loading stress factors.

The significant increase in the values of Hb and EOF in the control goats not administered AA immediately and 3 days after transportation was evidence of excess erythrocyte destruction. This showed that road transportation of the goats for 12 hr during the hot-dry season in the experimental zone was stressful and had negative effects on the RBCs of the animals for at least 3 days after the journey. Thus, for the first time, the potential use of EOF as a diagnostic tool in road transportation stress has been demonstrated in goats in the present study. The results of the present study on the post-transportation responses of RBCs agree with the findings that increased RBC destruction is due to exhaustion and tissue damage after a prolonged stress or exhaustive exercise in humans and animals [2, 14, 15, 20, 29, 39]. Factors responsible for such increase in hemolysis in the present study may be an increase in mechanical destruction of erythrocytes as a result of physical compression of erythrocytes against the walls of blood vessels and capillaries during handling, loading and maintenance of balance during transportation, a high AT and a high RH. These factors are known to cause a loss in erythrocytes elasticity and a decay of their energy metabolism, leading to erythrocyte damage [29, 31, 34, 39]. Other factors responsible for the increase in hemolysis include increased consumption of oxygen by muscles under stress, changes in blood pH [38], dehydration, hemoconcentration due to water loss from vessels, sweating, release of splenic RBCs [39], changes in RBC morphology, increments in intracellular Ca^{2+} due to α-adrenergic stimulation and tissue damage by xanthine oxidase [16, 35, 36, 40].

The fact that the values of Hb and EOF obtained in the goats administered AA immediately after transportation were not significantly different (P>0.05) from the post-loading values and that the post-transportation values returned to the pre-loading values 12 hr after the journey demonstrated, for the first time, that AA protected the erythrocytes of the goats transported for 12 hr under unfavorable climatic conditions from hemolysis. Furthermore, AA abolished the adverse effects of unloading and post-transportation stress on the goats, which are known to be stressful and associated with a new bout of cortisol release [7, 10, 27]. The mechanism by which AA protected the erythrocytes from damage during transportation may be AA's continuous inhibition of cortisol, the chief hormone of stress, and by donation of free molecules of hydrogen, which detoxify ROS, which reportedly increase in concentration with journey duration or stress [7, 21, 25]. AA reduces the capacity of O_2 consumption, decreases heat load and increases heat loss during stress [11, 21, 25], and this may also protect against erythrocyte damage during transportation. This is a desirable factor, especially during the hot-dry period when the demand for O_2 is high, and an increase in cell O_2 consumption is associated with a rise in the production of harmful ROS [15, 25, 29, 39]. The results obtained in the experimental goats 12 hr and 3 days post-transportation indicated that AA may reduce the release of factors like LDH and xanthine oxidase known to be responsible for exhaustion and tissue damage after prolonged stress or strenuous muscular activity [16].

The results for MCV and MCH obtained in the control goats indicated that loading and transportation decreased the volume and average Hb content in regard to individual RBCs. The MCH values recorded in the present study were significantly (P<0.01) lower than the average value of 31.0 *p*g obtained in healthy and non-transported goats [17]. Although the MCV values fell within the reference range of 18 to 34 fl [37], the significant decrease in the MCV values post-loading indicated that loading and transportation stress, apparently, destroyed the older and stable erythrocytes known to have larger sizes [39]. Higher MCV values are associated with larger, healthier and stable erythrocytes [34]. The results obtained in the AA-treated goats suggested that AA protected the erythrocytes from damage and hemolysis and that as a consequence, the population of older erythrocytes remained intact and stable after transportation. The present results agree with those of Tauler *et al.* [39] in humans administered AA and subjected to exhaustive exercises.

The fact that MCHC significantly increased after loading and transportation confirmed the increase in Hb concentration observed in the control goats. The increase in MCHC obtained post-transportation was higher (p<0.05) than the average MCHC value of 32.9 g/d*l* obtained in healthy non-transported goats [17]. The Hb index suggests that erythrocytes are normal (normochromic) if equal to one, hypochromic if less than one, which indicates a lower concentration of Hb in the erythrocytes, hyperchromic if higher than one [37]. The results for the Hb index obtained in the present study, therefore, indicated that loading and transportation

resulted in hypochromic RBCs (lower concentration of Hb), especially in the control goats. However, administration of AA relatively sustained the RBCs in a normochromic status, which suggested less cell damage.

Overall, AA mobilized all the defense and compensatory mechanisms of the goats and protected the erythrocytes from the deleterious effects of road transportation stress. In conclusion, the results of this study demonstrated that 12 hr of road transportation during the hot-dry season had deleterious effects on the RBC, and administration of AA 30–40 min prior to transportation ameliorated the stresses induced by handling, loading, transportation and the concomitant effect of high AT and RH on the erythrocytes of goats. In addition, we have suggested the potential use of EOF as one of the diagnostic tools for road transportation stress.

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