AMELIORATIVE EFFECT OF ASCORBIC ACID ON SOME BIOCHEMICAL PARAMETERS AND CORTISOL CONCENTRATION OF WEST AFRICAN DWARF BUCKS TRANSPORTED BY ROAD

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Abstract

This study was conducted to evaluate the ameliorative effect of ascorbic acid on some biochemical parameters and cortisol concentration of West African Dwarf (WAD) bucks transported by road for eight (8) hours. Twenty eight (28) WAD bucks were used for this study. The animals were randomly allocated into four groups (A to D) of seven (7) each. On the day of transportation, animals in groups A and C were orally administered with vitamin C at the dose of 250mg/kg dissolved in 10ml of sterile water while animals in groups B and D were individually and orally administered with only sterile water. Animals in groups A and B were transported while animals in groups C and D were not involved in the transportation but act as positive and negative control respectively. Blood samples were collected before transportation, after loading, immediately after transportation and three days post transportation and serum was harvested for the determination of biochemical parameters and cortisol concentration. The results showed that there were no significant differences in total protein, albumin, ALP, AST and ALT concentrations obtained pre-load, and post transportation in all the groups, but the values of AST and ALT differed significantly ($p<0.05$) after loading. The values of biochemical parameters like AST obtained three days post transportation were higher than their pre-transportation values. Cortisol concentration increased in all the groups post transportation, and the value in group B was significantly ($p<0.05$) higher than groups C and A.

In conclusion, ascorbic acid administration prior to 8 h of road transportation in WAD bucks decreased the adverse effects of the transportation on biochemical parameter and cortisol concentration. The ameliorative effect of ascorbic acid was particularly observed after loading and immediately after transportation.

Key words: *Road transportation, ascorbic acid, WAD bucks, biochemical parameters, cortisol concentration.*

INTRODUCTION

Goats are among the main meat-producing animals in developing countries and one of the choicest meats with huge domestic demand in Nigeria (Bourn *et al.,* 1994). Besides meat, goats provide products like milk, skin and manure. Goats do not only supply protein but also serve as a source of livelihood, generating income for the family (Anaeto *et al*., 2010).

FAO (2011) predicted that the demand for livestock products will be doubled in the next 20 years due to world population increase, urbanization, and economic growth. This, therefore, provides an excellent opportunity for goat producers and marketers.

Animal transportation means the intentional movement of animals by one or more means of transport which may include operations like loading, unloading, transfer and rest, until the unloading of the animals at the place of destination is completed (Animal Transport Act, 1429/2006 of the Ministry of Agriculture and Forestry, England). The most common means of transport for all livestock species in many countries of the world, including Nigeria, is by road (Minka and Ayo, 2013; Adenkola *et al*., 2009). In Nigeria, food animals are transported often without observation of any welfare order from European Union recommendations on the welfare of the Animal Transport Order (Anon, 1991).

These animals are transported in vehicles meant for transportation of goods and not specifically for the transportation of animals; such vehicles include open trailers, trucks, pick-ups and even buses together with human passengers (Adenkola and Okoro, 2015). These animals are often exposed to a variety of environmental extraneous stimuli, or stress factors (Frazer and Brown, 1990), and thus, road transportation of livestock in our environment is very stressful to animals (Adenkola and Ayo, 2009; Adenkola *et al*., 2009). Livestock are also transported for replacement of old stocks with new ones, exhibitions, fair, sports competitions, seasonal tourism, taming, reproduction and health status (Giovagnoli *et al.,* 2002). Transportation stress has remarkable physiological effects such as increased adrenal cortical activity, decreased immunity, increased morbidity and mortality due to infectious diseases, oxidative stress, which impairs antioxidant status of the body (Tajik *et al*., 2016). Thus, there is a need to administer an antioxidant exogenously to boost the endogenous source, especially during transportation, which usually causes stress. Ascorbic acid has been demonstrated to increase body resistance to environmental stress (Adenkola *et al*., 2016) by reducing the synthesis and secretion of corticosteroids, thus, reducing the negative effect of stress. Ascorbic acid (AA) is a water-soluble antioxidant, readily metabolized by many animals and humans (Adenkola and Onyeberechi, 2015) and is affordable and available.

Information on transportation of goats by road in the middle belt region of Nigeria is scanty. This research is therefore, aimed at investigating the ameliorative effect of ascorbic acid administration on some biochemical parameter and cortisol level in West African dwarf goats transported by road.

Materials and Methods

Study Area

The study was conducted at the Small Ruminant Unit of the Federal University of Agriculture Teaching and Research Farm Makurdi, Benue State, Nigeria. Makurdi is located in Latitude 6 – 8 ^{0} N and Longitude 6 – 10 ^{0} E. The area is warm with a minimum temperature range of 17.3 – 24.5 ^{0}C and a maximum temperature range of 26.5 - 42^{0}C with annual rainfall of 1,317 – 1,323 mm which spans between 6 - 7months, while the relative humidity is between 47- 85% (Adenkola and Okoro, 2015).

Experimental Animals and management

Twenty eight (28) West African Dwarf male goats, eight months to one year old served as the subjects of this study. The goats were sourced from Makurdi metropolis. They were reared under the semi intensive management system in the pens in the Small Ruminant Unit of the University farm. The building has a long corridor of about 1m long with each pen measuring 285 x 285cm for animals on both sides of the corridor. The pen has a large wide windows measuring 180 x 126cm with a wire mesh for natural ventilation. The goats were not restrained inside the pen. The goats were allowed to acclimatize for a period of two weeks. During the period of acclimatization before the commencement of the experiment, the goats were screened for common diseases and were treated prophylactically against ecto- and endoparasites. Thereafter, the goats were individually vaccinated using National Veterinary Research Institute, Vom Peste des petits ruminants (PPR) vaccine against PPR and were ear-tagged to enable identification.

Experimental Protocol/ Design

Twenty eight (28) selected WAD bucks were randomly allotted into four groups of seven bucks each (Group A– D). Bucks belonging to each group were identified and numbered with plastic ear-tags. The grouping of the bucks was done as presented in Table 1:

Table 1: Grouping of the experimental animals

S/N	Group	Antioxidant (ascorbic acid) administration	Animal involved in transportation
01	A (n=7)	YES	YES
02	B (n=7)	NO, distilled water only	YES
03	C (n=7)	YES	NO
04	D (n=7) (negative control)	NO, distilled water only	NO

Transportation of animals

On the day of transportation;

- Group A and C (n = 7 each) animals were orally administered with ascorbic acid at 250 mg/kg (Chervyakov *et al.*, 1977) dissolved in 10 ml of water,

- Group B and D (n =7 each) were given only 10 ml of sterile water.

The administration was made between 30 to 40 minutes before loading the goats into the vehicle. Food and water was withdrawn 12 h before the journey and throughout the journey period (Welfare of Animals Transport Order, 1997). The vehicle travelled along Makurdi - Otukpa road from Federal University of Agriculture Makurdi Teaching and Research Farm on tarred smooth and rough road for 8 hours at the speed of 40-50 km/h covering a total of about 400 km and back to the starting point. After completing the journey, the goats were unloaded at the spot where they were loaded. The animals were fed and watered as they had been prior to the journey.

Vehicle design

A standard Ford bus, popularly used in the Middle Belt region of Nigeria for transportation of livestock was used to transport the bucks. The inner compartment of the vehicle measured 3.63 m long, 1.35 m wide and 1.7 m high. The side walls of the vehicle from the floor to the roof were completely covered with corrugated aluminium sheets, which were smooth with no protrusion of sharp edge and 'with a window, which provided for adequate ventilation. Each window measured 1.02 by 0.51 m on both sides of the vehicle and was at the height of about 0.71m from the floor. A door, which measured 1.3 m by 1.59 m was provided at the rear end of the vehicle. Other transportation procedures were carried out in accordance with the standard guidelines governing the welfare of livestock during road transportation (Warris, 1996). They were made to stand inside the vehicle in rows without any form of restraint. The journey commenced at 9:00 am on the day of transportation.

Blood Sample Collections

Blood samples were taken; (i) 7 am in the morning a day before transportation, (ii) post loading, (iii) immediately after transportation and (iv) three (3) days post-transportation.

Ten millimeters of blood was taken aseptically from the jugular vein using a 10 ml syringe and 21 gauge x $1^1/2$ inch sterile needles from each animal and was poured immediately inside a sample bottle. The blood samples were centrifuged and the serum was harvested and stored at a temperature of -20^{o}C for the determination of biochemical parameters and cortisol level.

Sample Analysis

Determination of Serum Biochemical Parameters

Serum biochemical parameters such as total proteins, albumin, and globulins were analysed by the method of Gomall *et al.* (1949) while alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase were estimated calorimetrically using Randox reagent diagnostic kits (Reitman and Frankel, 1957).

Cortisol Concentration was determined using ELISA (Enzyme linked immune-sorbent assay) (Diagnostic automation Inc) method based on the principle of cortisol (antigen) in the sample connecting the horse radish peroxidase-cortisol.

Statistical Analysis

The data obtained was presented in tables. Data was subjected to one way analysis of variance (ANOVA) and LSD post hoc analysis using SPSS version 20. Values of $P \leq 0.05$ were considered significant.

Results

Effects of ascorbic acid administration, loading and road transportation on biochemical parameters in West

African Dwarf goats are shown in table 2 to 5 respectively. The results of the measurement of the serum concentrations of total proteins, albumin, globulins as well as alkaline phosphatase (ALP), aspartate amino transferase (AST) and alanine amino transferase (ALT) of WAD goats before transportation (**Table 2)** were no significantly ($p>0.05$) different among the groups. However, the values of total protein (7.22 ± 0.08g/dl) obtained in group D was higher than 6.00 ± 0.66g/dl obtained in group B. AST values were found to be highest in group A (101.36 ± 2.59 I.U/L) and lowest in group D (96.38 ± 2.69 I.U/L) but these values were not significant ($p>0.05$) statistically. Also, ALT values were highest in group B (35.36 ± 8.68 I.U/l) and lowest in group C (27.46 ± 1.73 I.U/L). Alkaline phosphatase (ALP) values were highest in group A with the mean value of 40.28 ± 7.39 I.U/L and lowest in group B (29.62 ± 5.26 I.U/L). These values were not significantly ($p>0.05$) different. Total protein values decreased slightly in all the groups after loading of the animals (**Table 3),** but the values were not significantly ($p>0.05$) different from the base-line values obtained before transportation. Total protein values later increased slightly in all the groups with animals in group B having the highest value of 5.98 ± 0.54 I.U/L immediately after transportation. Three days post transportation, the concentration of total protein in all the groups did not vary significantly ($p>0.05$). However, albumin values were significantly ($p<0.05$) higher in group A (2.90 ± 0.14 g/dl) and D (2.84 ± 0.09 g/dl) when compared with the mean value of albumin obtained in group B (2.52 ± 0.06g/dl) three days after transportation. The values of alkaline phosphatase (ALP) after loading in group A and C were 21.92 ± 4.86 and 23.04 ± 5.71 while group B and D had values of 19.52 ± 3.95 and 21.26 ± 4.81 I.U/L. These values were not significantly ($p>0.05$) different statistically. The ALP values in group B and D decreased after loading and immediately after transportation with mean values of 15.20 ± 0.67 I.U/L and 16.38 ± 0.97 I.U/L. However, the mean value of ALP recorded three days post transportation increased slightly when compared with the values recorded after loading and immediately after transportation. The aspartate aminotransferase (AST) values increased from values of 99.36 ± 1.62 before transportation to 136.58±9.76 I.U/L recorded immediately after transportation in group B. The mean values of AST recorded in group A, C and D also increased after loading and immediately after transportation. However, these values did not differ significantly ($p<0.05$). Three days post transportation, AST mean values obtained were 110.64 ± 3.36, 110.56 ± 4.63, 116.66 ± 3.71 and 114.54 ± 4.19 I.U/Lin groups A, B, C and D respectively. The above values were not significantly ($p>0.05$) different from each other but were higher than the baseline values obtained pre transportation. After loading of the goats, alanine amino transferase (ALT) values rose from the pre-transportation values of 27.86 ± 1.25 to 28.68 ± 0.28 in group A, 27.46 ±1.73 to 27.62 ± 1.03 I.U/L in group C while ALT mean value in group B decreased from 35.36 ± 8.68 to 30.62 ± 0.44 I.U/L, and 32.04 ± 1.14 to 26.34 ± 0.35 I.U/Lin group D after loading. The mean ALT values recorded immediately after transportation was higher in the non-ascorbic acid supplemented groups (B and D) than the ascorbic acid supplemented group (A and C). However, there were no significant ($p>0.05$) differences in the values recorded in the various groups irrespective of their treatments immediately after transportation. ALT values three days after transportation in group A remain almost the same as values before transportation while ALT values in groups B, C and D were lower than their baseline values.

Serum cortisol concentration **(table 6)** obtained before transportation in all the goats were not statistically significant ($p>0.05$) across the groups, although group A had the highest cortisol concentration of 39.59 ± 13.61 while group B had the lowest concentration of cortisol with the value of 37.42 ± 4.60 ng/ml. The concentration of cortisol gradually increased after loading in all the groups but the increase was higher ($p<0.05$) in group B and D with the values of 55.10 ± 5.24 ng/ml and 46.40 ± 2.63 ng/ml as against 47.56 ± 2.49 ng/ml and 43.84 ± 2.81 ng/ml obtained in groups A and C, respectively. There was significant ($p<0.05$) difference in the concentration of cortisol obtained in groups B and C post loading. After transportation, we observed that, there was significant ($p<0.05$) increase in the concentration of cortisol in all the groups when compared with the initial concentration obtained before and after loading. The cortisol concentration was higher in non-ascorbic acid supplemented groups. The concentration of cortisol obtained in all the groups did not vary significantly ($p>0.05$) three days post transportation. Furthermore, cortisol concentration values obtained three days post transport was lower than values obtained after transportation and loading.

Table 2: Serum biochemical parameters of goats before transportation (mean ± SEM)

PARAMETERS	Group A	Group B	Group C	Group D
Total protein (g/dL)	6.28 ± 0.64	6.00 ± 0.66	6.78 ± 0.24	7.22 ± 0.08
Albumin (g/dL)	2.62 ± 0.18	2.42 ± 0.15	2.56 ± 0.16	2.88 ± 0.26
Globulin (g/dL)	3.66 ± 0.55	3.58 ± 0.62	4.22 ± 0.38	4.34 ± 0.26
Alkaline phosphatase (I.U/L)	40.28 ± 7.39	29.62 ± 5.26	35.86 ± 8.87	39.78 ± 10.01
Aspartate amino transferase (I.U/L)	101.36 ± 2.59	99.36 ± 1.62	98.16 ± 2.51	96.38 ± 2.69
Alanine amino transferase (I.U/L)	27.86 ± 1.25	35.36 ± 8.68	27.46 ± 1.73	32.04 ± 1.14

Values along the same row are not significantly different ($p>0.05$)

Table 3: Effect of ascorbic acid administration on serum biochemical parameters of WAD goats after loading (Mean ± SEM)

PARAMETERS	Group A	Group B	Group C	Group D

Total protein (g/dL)	5.16 ± 0.31	5.72 ± 0.28	5.26 ± 0.19	5.02 ± 0.30
Albumin (g/dL)	2.06 ± 0.11	2.34 ± 0.11	2.12 ± 0.18	2.52 ± 0.18
Globulin (g/dL)	3.10 ± 0.32	3.38 ± 0.25	3.14 ± 0.17	2.50 ± 0.47
Alkaline phosphatase (I.U/L)	19.14 ± 3.95	15.20 ± 0.67	20.24 ± 4.78	16.38 ± 0.97
Aspartate amino transferase (I.U/L)	122.66 ± 5.64	136.00 ± 11.76	115.90 ± 7.89	119.16 ± 1.17
Alanine amino transferase (I.U/L)	28.68 ± 0.28^{ad}	30.62 ± 0.44^{b}	26.34 ± 0.35^{c}	27.62 ± 1.03^{ad}

Values along the same row with different alphabet superscripts are significantly different ($p<0.05$)

Table 4: Effect of ascorbic acid administration on serum biochemical parameters of WAD goats immediately after transportation (mean ±SEM)

PARAMETERS	Group A	Group B	Group C	Group D
Total protein (g/dL)	5.56 ± 0.54	5.98 ± 0.54	5.46 ± 0.51	5.66 ± 0.28
Albumin (g/dL)	2.46 ± 0.25	2.26 ± 0.18	2.74 ± 0.15	2.50 ± 0.21
Globulin (g/dL)	3.10 ± 0.75	3.72 ± 0.58	2.72 ± 0.51	3.16 ± 0.33

Alkaline phosphatase (I.U/L)	21.92 ± 4.86	19.52 ± 3.95	23.04 ± 5.71	21.26 ± 4.81
Aspartate amino transferase (I.U/L)	122.78 ± 9.58	136.58 ± 9.76	111.48 ± 15.08	119.30 ± 4.8
Alanine amino transferase (I.U/L)	36.42 ± 1.58	37.30 ± 1.48	34.56 ± 1.51	36.92 ± 0.7

Values along the same row are not significantly different (p>0.05)

Table 5: Serum biochemical parameters of West African dwarf goats three (3) days after transportation (Mean ±SEM)

PARAMETERS	**Group A**	**Group B**	**Group C**	**Group D**
Total protein (g/dL)	6.36 ± 0.18	5.94 ± 0.12	6.16 ± 0.12	6.24 ± 0.26
Albumin (g/dL)	2.90 ± 0.14^{a}	2.52 ± 0.06^{b}	2.64 ± 0.04	2.84 ± 0.09^{d}
Globulin (g/dL)	3.46 ± 0.20	3.42 ± 0.14	3.52 ± 0.12	3.40 ± 0.25
Alkaline phosphatase (I.U/L)	22.92 ± 1.54^{a}	25.64 ± 2.00^{bc}	25.70 ± 1.86^{bc}	28.00 ± 0.80^{d}
Aspartate amino transferase (I.U/L)	110.64 ± 3.36	110.56 ± 4.63	116.66 ± 3.71	114.54 ± 4.19
Alanine amino transferase (I.U/L	27.02 ± 0.65^{ab}	25.98 ± 1.16^{ab}	24.08 ± 0.74^{cd}	23.74 ± 0.55^{cd}

Values along the same row with different superscript are significantly different ($p<0.05$)

Table 6: Serum Cortisol concentration (ng/ml) of West African Dwarf goats at different time intervals (Mean ±SEM)

TIME	Group A	Group B	Group C	Group D
Before transport	39.59 ± 13.61	37.42 ± 4.60	38.22 ± 3.09	38.58 ± 1.98
After loading	47.56 ± 2.49[a]	55.10 ± 5.24[b]	43.84 ± 2.81[c]	46.40 ± 2.63[a]
After Transport	61.32 ± 7.82[bcd]	74.34 ± 3.25[b]	56.00 ± 11.43[c]	64.14 ± 8.61[d]
3days post Transport	45.14 ± 5.42	49.78 ± 3.28	43.48 ± 3.13	46.46 ± 2.01

Values along the same row are not significantly different ($p>0.05$)

DISCUSSION

The non-significant increase in total protein concentration observed in all groups in this study agrees with the observations of Adenkola *et al*. (2011) in pigs but in contrast to the observations of Rajesh *et al*. (2003) in sheep and Haydaideoglu *et al.* (2017) in horses. The later authors reported a significant ($p<0.05$) increase in total protein post transportation. The differences in results might be due to the duration of journey, season of research and species of animals used. The significant increase ($p<0.05$) in transaminase enzymes (AST, ALT) observed in non-ascorbic acid supplemented groups immediately after transportation may be due to enormous free radicals produced which, might have caused the secretions of the enzyme by the hepatocytes. However, an increase in the values of these enzymes 3days post-transportation is an indication of minimal to severe muscular and liver insults especially in group B goats, where we observed the increase in the elevation of these enzymes. The results in gosats are similar to that obtained by Adenkola *et al*. (2011) in pigs transported for 8h during harmattan season, where an increase in transaminase enzymes and ALP were noted. The maintenance of the transaminase enzyme values in the ascorbic acid supplemented groups is an indication of the protective effect of AA in scavenging free radicals and ameliorating the stressful condition (Minka and Ayo, 2013). Handling and transportation have been shown to elicit an increase in adrenal cortex responses viewed as stressors (Giammarco *et al*., 2012). In the present study, serum cortisol increased significantly apparently due to the activation of the response system as seen immediately after loading and transportation in non-ascorbic acid supplemented groups ($p<0.05$). The above finding in this study agrees with the previous studies of Tajik *et al*. (2016); Kannan *et al*. (2000) and Idrus *et al.* (2010) who reported a significant increase/elevation in serum cortisol concentration after loading and transportation in goats. Also Giammarco *et al*. (2012) and Haydardeoglu *et al*. (2017) reported an increase in cortisol concentration after transportation in rabbits and horses respectively. The current study showed that 8 h transportation of WAD goats caused 3-fold increase in serum cortisol. The concentration of cortisol obtained three days post transportation was lower than the value obtained immediately after transportation but did not return to the baseline value obtained before transportation. In contrast to this result, Sanhouri *et al*. (1991) and Nwe *et al*. (1996) reported that cortisol concentrations in goats began to decrease immediately after transportation and reached baseline level 3 hours after transportation. Kannan *et al*. (2000) and Saeb *et al*. (2010) also reported that serum cortisol in goats began to decrease 1 hour after transportation. The difference between species, difference in time trend of sampling and different transportation conditions could be the possible cause. Furthermore, the rate of decline in serum cortisol levels following peak concentrations in goats may vary according to the type and duration of stressors (Sanhouri *et al*., 1991). The fact that only male WAD goats were used for this study could be another reason for the difference as male animals are believed to be more excited and stress sensitive than female animals (Okeudo and Moss, 2005). The moderate increase in the serum cortisol concentration of the AA-supplemented groups showed the inhibitory role of AA on circulating corticosteroids in group A and C following transport stress. This result is in line with the report of Belge *et al*. (2003) and Adenkola and Angani (2017) who reported that adverse effects of stress could be mitigated by AA supplementation in chicken.

In conclusion, eight (8) hours of road transportation of WAD goats was stressful which was evident in the increased level of circulating cortisol after loading and immediately after transportation. Administration of ascorbic acid prior to loading and transportation of the goats might have reduced the level of circulating cortisol. Thus, administration of ascorbic acid (Vitamin C) before transportation of livestock is recommended.

Acknowledgement

The authors wish to acknowledge Mr. Vincent Apev of Veterinary Physiology and Biochemistry Department, University of Agriculture, Makurdi, for taking us through the laboratory procedures.

Competing interests

Authors have declared that no competing interests exist.

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