International Scholars Journals

African Journal of Pig Farming ISSN 2375-0731 Vol. 4 (8), pp. 001-005, August, 2016. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Effect of road transportation on erythrocyte osmotic fragility of pigs administered ascorbic acid during the harmattan season in Zaria, Nigeria

A. Y. Adenkola[1*] and J. O. Ayo[2]

[1]Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria.

[2]Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

Accepted 15 December, 2016

This study was conducted with the aim of investigating the effect of eight hours road transportation on pigs administered ascorbic acid (AA) during the harmattan season. Twenty nine pigs were used for the study, seventeen pigs were administered with AA at a dose of 250 mg/kg *per os* and served as experimental animals, while 13 others each administered orally with sterile water served as control animals. The animals were then transported for 8 h at a speed of 40 - 50 km/h covering a distance of 260 km. Blood samples were taken early in the morning a day before transportation, immediately after (within 30 min) and a week after transportation. Thereafter, erythrocyte osmotic fragility (EOF) was determined using a standard method. There was a statistically significant (P < 0.05) difference in percent haemolysis recorded at NaCl concentration of 0.30, 0.40 and 0.60% between experimental and control pigs immediately after transportation. The results of the present study indicated that measuring osmotic resistance of erythrocytes could be used as a determinant of oxidative stress in pigs transported by road.

Key words: Ascorbic acid, erythrocyte osmotic fragility, harmattan season, pigs, road transportation.

INTRODUCTION

The most common means of transportation for all live-stock species including the pig all over the world and Nigeria inclusive, is by road (Ayo and Oladele, 1996; Giovagnoli et al., 2002; Adenkola et al., 2007; Buckham Sporer et al., 2008), which is often stressful to the animals (Von Borell, 2001; Vecerek et al., 2006; Pineiro et al., 2007; Buckham Sporer et al., 2008). Stress factors have been shown to cause oxidative stress and impair the activity of the antioxidant, vitamin C or ascorbic acid (AA) *in vivo* (Halliwell, 1996; Sahin et al., 2001) and the depletion of some of this antioxidant systems could increase the vulnerability of tissues and of cellular com-ponents reactive oxidation species (Piccione et al., 2007).

Free radicals are known to play a vital role in tissue damage, and they have been demonstrated to have adverse effects on erythrocyte (Sumikawa et al., 1993; Avellini et al., 1995) and may be formed in the course of physiological and pathological processes in aerobic organism, and the combination of this with cellular components may result in cellular dysfunction (Piccione et al., 2007) if free radical “quencher” is not available to terminate the reactions (McCord, 1985; Akinwande and Adebule, 2003). Antioxidant supplementation, therefore, has been shown to be beneficial in attenuating the adverse effect of environmental stress (Kafri and Cherry, 1984) and stress-induced tissue damage (Sen, 2001). There is a serious concern about how long pigs should be transported without being offered food and water. The current UK legislation [Welfare of Animals (Transport) Order, 1997] states that pigs can be transported for a maximum of eight hours after which they must be unloaded, fed, watered and rested for 24 h before any further journey (Brown et al., 1999). There is therefore a need to investigate the effect of long journeys on the erythrocyte membrane of transported pigs. There is paucity of information on the adverse effects of road

*Corresponding author. E-mail: aadenkola@yahoo.com. Tel.: +234-805-4977696.

transportation on erythrocyte osmotic fragility of pigs, especially during the harmattan season, described to be the most stressful of all the three seasons (hot-dry, rainy and harmattan) prevailing in the Northern Guinea Savannah zone of Nigeria (Igono et al., 1982).

The aim of the present study was to investigate the effect of AA on EOF in pigs transported by road for 8 h during the harmattan season.

MATERIALS AND METHODS

Experimental site and meteorological conditions

The experiment was performed at the Livestock Research Pen, Faculty of Veterinary Medicine, Ahmadu Bello, University, Samaru, Zaria (11° 10' N, 07° 38' E), located in the Northern Guinea Savannah zone of Nigeria during the harmattan season. Harmattan season in Nigeria occurs between late-November and early- March (Igono et al., 1982; Oladele et al., 2003). This zone is characterised by intensive livestock marketing and transportation. During the study, wet and dry-bulb temperatures (DBT) were determined six times twice in a week pre-transportation and for three consecutive days post-transportation at 06:00, 13:00 and 18:00 h at the experimental site using dry- and wet -bulb thermometers (BRANNAN®, England), and relative humidity (RH) was calculated using the manufacturer's standard manual.

Experimental animals and management

Twenty nine local pigs comprising males and non- pregnant, non-nursing females of different age groups, ranging from 9 to 12 months and were obtained in Zaria and its environs. The pigs were kept in a standard communal pen, made of concrete floor and iron walls with asbestos roofing. The pen measured 7.50 x 2.55 m with half the length to the roof without block work, which provided for adequate ventilation. The pigs were allowed to roam freely in the pen. They were kept under an intensive system of management and were fed with maize offal, brewers waste, yam peels, and water was given *ad libitum*. The pigs were pre-conditioned for two weeks before the commencement of the experiment. During the period, they were screened for haemoparasites and endoparasites by taking their blood and faecal samples for analyses. Pigs found to be infected with haemoparasite were treated using oxytetracycline (KEPRO B. V® , Holland) at the dose of 20 mg/kg by deep intra-muscular route and those infested with endoparasites were treated with Thiabendazole (M.S.D AGVET®, U.S.A.) at the dose rate of 25 mg/kg, *per os* respectively.

Experimental design, transportation of animals and blood sample collection

On the experimental day, 17 pigs were each administered orally with AA (Juhel®, Enugu, Nigeria) at 250 mg/kg (Chervyakov et al., 1977) dissolved in 20 ml of water, while 12 pigs which served as controls were each administered 20 ml of sterile water orally. The drugs were administered immediately before loading the pigs into the vehicle. Food and water were withdrawn 12 h before and throughout the journey. The vehicle traveled from the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria on tarred road along Zaria - Jos road to Pambegua (10°4' N, 08° 16' E) and back to Zaria (11°10' N, 07° 38' E) covering a total distance of 260 km at a speed range of 40 - 50 km/h. The journey lasted eight hours, including brief stop-overs at police checkpoints. After completing the journey, the pigs were unloaded at the original loading point, fed and watered.

Blood samples were taken early in the morning a day before transportation, immediately and a week after transportation. Five milliliters of blood was drawn aseptically from each animal via the anterior vena cava using a 10 ml syringe and 18 gauge x $1^{1}/_{2}$ inch sterile needle. The blood was immediately poured into sample bottles, containing the anticoagulant, disodium salt of ethylene diaminetetra-acetic acid at the rate of 2 mg/ml of blood (Oyewale, 1992). After collection, the samples were transferred to Physiology Research Laboratory, Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, where erythrocyte osmotic fragility (EOF) test was carried out as described by Faulkner and King (1970).

Erythrocyte osmotic fragility determination

Sodium chloride (NaCl) solution was prepared according to Faulkner and King (1970) in volume of 500 ml for each of the samples in concentrations ranging from 0.05 to 0.85% at pH 7.4. A set of 10 test tubes, each containing 10 ml of NaCl solution of concentrations, ranging from 0.05 to 0.85%, were arranged serially in a test tube rack. One set was used to analyse each sample. The test tubes were labeled with corresponding NaCl concentrations. One ml pipette was used to transfer exactly 0.02 ml of blood sample into each of the ten test tubes. Mixing was performed by gently inverting the test tubes for about 5 times. The test tubes were allowed to stand at room temperature (26 - 27°C) for 30 min. The contents of the test tubes were maintained at pH 7.4. Thereafter, the contents of the test tubes were re-mixed and centri-fuged at 1,500 x g for 15 min. The supernatant of each test tube was transferred into a glass cuvette. The concentration of haemo-globin in the supernatant solution was measured at 540 nm using a spectrophotometer (SPECTRONIC-20, Philip Harris Limited®, Shenstone, UK) by reading the absorbance. The same procedure was repeated for every blood sample of each pig used for the study. The percent haemolysis was calculated using the formula (Faulkner and King, 1970).

$$\frac{\text{Optical density of test}}{\text{Optical density of distilled water}} \times 100 = \text{Percent haemolysis}$$

Erythrocyte osmotic fragility curve was obtained by plotting percent haemolysis against the saline concentrations.

Statistical analysis

All data obtained were subjected to statistical analysis using Student's *t*-test. Data were expressed as mean ± standard error of mean. Values of $P < 0.05$ were considered significant.

RESULTS

Meteorological Data

The meteorological data from the study period is shown in Table 1 and 2. The period was characterized by relatively low minimum AT of 19.0 ± 3.1°C and low maximum AT of 23.3 ± 0.7°C. The DBT value obtained during the recordings was 19.3 ± 2.7°C. The harmattan season was characterized by relatively high humidity of 68.0 ± 8.5%. The wind direction was North-east. The meteorological data during the post-transportation period were similar to those obtained during the pre- transportation period ($P > 0.05$).

Table 1. Meteorological data from the study period pre-transportation.

Hour	Ambient Temperature (°C)			Relative Humidity (%)
	Minimum	Maximum	Dry-bulb	
06:00	13	24	14	85
13:00	23	24	23	60
18:00	21	22	21	59
Mean ± S.E.M	19.00 ± 3.1	23.33 ± 0.7	19.33 ± 2.7	68.00 ± 8.5

Table 2. Post-transportation meteorological data from the study period.

Hour	Ambient Temperature (°C)			Relative Humidity (%)
	Minimum	Maximum	Dry-bulb	
06:00	15	25	14	80
13:00	24	26	24	60
18:00	23	21	20	63
Mean ± S.E.M	20.67 ± 2.85	24.00 ± 1.53	19.33 ± 2.91	67.67 ± 6.23

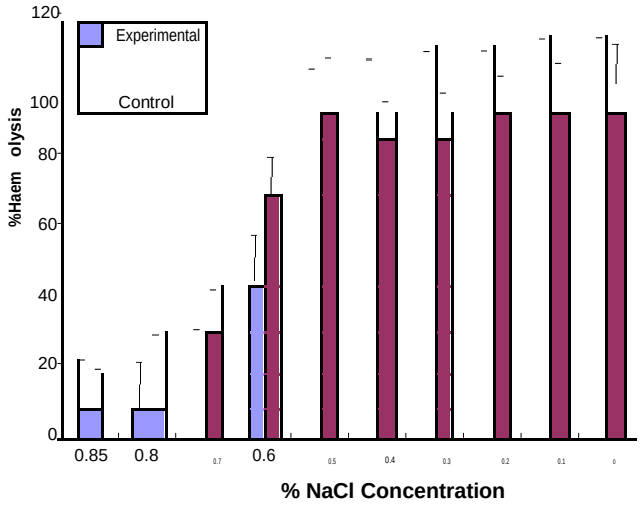

Figure 1. Effect of ascorbic acid on erythrocyte osmotic fragility before eight hours of road transportation in pigs.

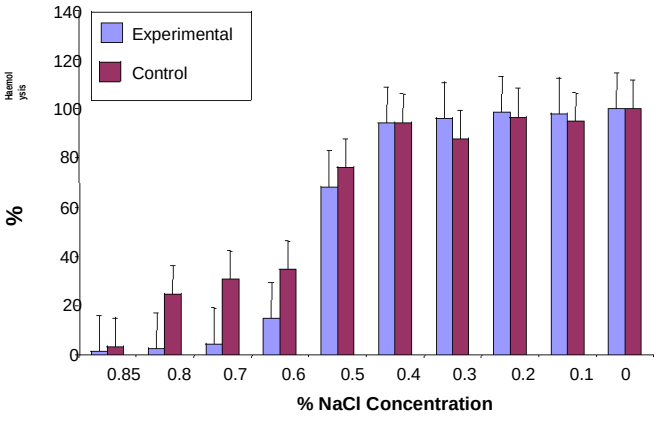

Figure 2. Effect of ascorbic acid on erythrocyte fragility immediately after eight hours of road transportation in pigs.

Effect of ascorbic acid administration on erythrocyte osmotic fragility of pigs transported by road for 8 h

The minimum haemolysis in the pigs was recorded at 0.80% NaCl solution in the experimental pigs, and the corresponding value in the control pigs was obtained at 0.85%. The maximum haemolysis of 99.5% was obtained at 0.10% in the experimental pigs, while this same was obtained at 0.50% in the control pigs. There was no significant ($P > 0.05$) difference in all the values recorded between the experimental and control pigs before road transportation (Figure 1). There was, however, a significant ($P < 0.05$) difference in percent haemolysis recorded at NaCl concentration of 0.30, 0.40 and 0.60% between the experimental and control pigs immediately after transportation (Figure 2). The minimum haemolysis obtained in experimental and control pigs occurred at 0.85% and the maximum value obtained in both experimental and control was at 0.20%. When compared to the pre-transportation value, a significant ($P < 0.05$) difference in percent haemolysis existed at 0.50% of NaCl concentration on day 7 after the journey and maximum haemolysis occurred at 0.1 and 0.5% in experimental and control pigs, respectively (Figure 3).

DISCUSSION

The results obtained in the present study show that pigs were subjected to a cold and dust-laden wind with high AT, characteristics of the harmattan season in the Northern Guinea Savannah zone of Nigeria. This observation agrees with that of Ayo et al. (1998a, b) . Meteorological data obtained during the present study agree with the previous findings that the harmattan season is thermally stressful to pigs (Fayomi et al., 2004; Adenkola and Ayo, 2006b). Heat stress impairs absorption of vitamins C, and thus increases the requirement of these vitamins, especially under heat stress condition (Naziroglu, 2000), when

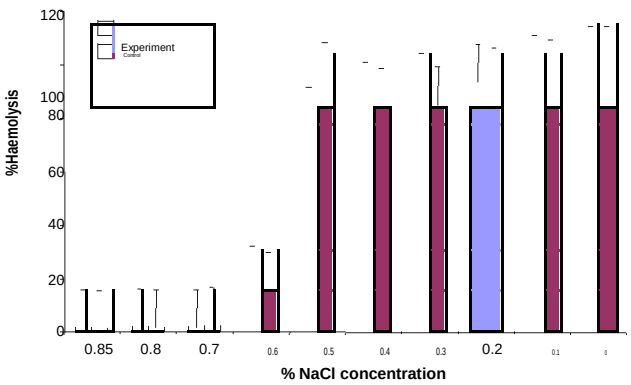

Figure 3. Effect of ascorbic acid on erythrocyte fragility on day 7 after eight hours of road transportation in pigs.

the concentration of antioxidant vitamins decreases, lipid peroxidation increases in the plasma and tissues leading to damage of cell membranes (Sahin, 2001).

The maximum haemolysis of erythrocytes occurred at the same % NaCl concentration and these result suggested that erythrocytes of these animals respond similarly to hypotonic solutions, before road transportation. There was no significant difference in EOF of both experimental and control pigs pre-transportation. Immediately after transportation, there was a decrease in percentage haemolysis at % NaCl concentration of 0.85, 0.80 and 0.70% which was more in the experimental pigs when compared to the controlled pigs. This is similar to the observation of Sumikawa et al. (1993) and Avellini et al. (1995) who noted that free radicals are known to play a vital role in tissue damage and has adverse effects on erythrocytes. It is a known fact that free radicals are generated in animals subjected to stress (Elsner, 1991; Halliwell, 1996) which is true in this study in which pigs were subjected to road transportation stress, although free radicals were not determined in this study. The result of the present study also agrees with the findings of Jain (1989), Deiss (1995) and Bei et al. (1996) that increased osmotic fragi-lity of erythrocytes due to oxidant stress was prevented with vitamin E and or vitamin C supplementation, and the resistance of erythrocyte was enhanced. The low percentage of haemolysis recorded in the experimental pigs were similar to the observations of Candan et al. (2002) who noted that AA improved erythrocyte fragility and led to reduced oxidative damage of erythrocytes. It has been established that AA ameliorates stress and the adverse effects of environmental conditions (Tauler et al., 2003; Adenkola and Ayo, 2006a b; Minka and Ayo, 2007). It is established in this study that administration of AA reduces the intensity of oxidant stress by enhancing the antioxidant defense mechanisms and suppression of this oxidant stress greatly minimized the destruction of erythrocytes. This could explain the observation in this study in which AA administered pigs had lower haemolysis than the pigs not-administered AA. AA is thus a free radical “quencher” that maintains the integrity of the erythrocyte cell membranes after road transportation in experimental pigs. The result of the present study indicates that measuring osmotic resistance of erythrocytes can be used as an indicator in determining oxidative stress in pigs transported by road.

Conclusion

Administration of AA prior to transportation of pigs is beneficial as it protects the integrity of the erythrocyte membranes in experimental pigs following road transportation, and thus may alleviate the risk of increased haemolysis due to road transportation stress in pigs during the harmattan season.

It is, therefore, recommended that AA be administered to pigs before transportation in order to reduce the adverse effects of road transportation stress on erythrocytes.

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