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Full Length Research Paper

Erythrocyte osmotic fragility of pigs administered ascorbic acid and transported by road for short-term duration during the harmattan season

A. Y. Adenkola[1]*, J. O. Ayo[2], A. K. B. Sackey[3] and A. B. Adelaiye[4]

[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.
[3]Department of Surgery and Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria.
[4]Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University Zaria, Nigeria.

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The experiment was carried out with the aim of investigating the effect of an antioxidant ascorbic acid on erythrocyte osmotic fragility of pigs transported by road for 4 h during the harmattan season. 16 pigs administered with ascorbic acid at the dose of 250 mg/kg per os and individually served as experimental animals and 13 others administered orally with sterile water were used as control animals. The animals were then transported for 4 h at a speed of 40 - 50 km/h covering a distance of 140 km. Blood samples for erythrocyte osmotic fragility determination which was done using standard procedure, were taken early in the morning a day before transportation, immediately after and a week after transportation. Erythrocyte osmotic fragility decreased significantly ($P < 0.05$) at NaCl concentration of 0.85, 0.80 and 0.70% in both experimental and control pigs following road transportation and the difference in the post-transportation values was higher ($P < 0.05$) in experimental compared to control pigs. The results indicated that ascorbic acid protected the integrity of the erythrocyte membrane in experimental pigs administered ascorbic acid following road transportation as demonstrated by lower percentage haemolysis immediately after road transportation and thus may alleviate the risk of increase in haemolysis due to road transportation stress in pigs during the harmattan season.

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

INTRODUCTION

In many parts of the world, including Nigeria, food animals are transported mainly by road. It has been established that road transportation is stressful to livestock (Rajion et al., 2001; Giovagnoli et al., 2002; Adenkola and Ayo, 2009). During stress, there is an increase in generation of reactive oxygen species (ROS) in the body to a level that overwhelms tissue antioxidant defense systems (Akinwande and Adebule, 2003; Powers and Jackson, 2008). The mechanism of damage involves lipid peroxidation which destroys cell membranes with the release of intracellular compoents, such as lysosomal enzymes, leading to further tissue damage (Demir et al., 2003). The ROS play a vital role in cellular and tissue damage (Tkaczyk and Vizek, 2007) and they have been demonstrated to have adverse effects on erythrocytes (Sumikawa et al., 1993; Avellini et al., 1995). The magnitude of stress in the body depends on the ability of the tissues to detoxify ROS (Williams et al., 2008) that is, their antioxidant defence. The ROS, including free radicals, initiate many reactions which are deleterious to body cells (Sudha et al., 2007), if the ROS "quencher" is not available to terminate the reactions (Wulf, 2002; Akinwande and Adebule, 2003). Antioxidant supplementation, therefore, has been shown to be beneficial in stress-induced tissue damage (Senturk, 2001; Adenkola

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

and Ayo, 2009). Vitamin C or ascorbic acid is a naturally occurring antioxidant (Sahin et al., 2001) and currently is the most widely used vitamin supplement throughout the world (Naidu, 2003). Ascorbic acid is an effective anti-oxidant because it has an important metabolic role as a result of its reducing properties and function as an electron carrier. It can give up 2 electrons, and it is converted to dehydro-L-ascorbic acid (Rice, 2000; Sahin et al., 2001). It has been established that ascorbic acid ameliorates heat stress and the adverse effects of stressful environmental conditions (Tauler et al., 2003; Adenkola and Ayo, 2006; Ayo et al., 2006; Minka and Ayo, 2007). The aim of the present study was to investigate the effect of ascorbic acid administration on the erythrocyte osmotic fragility of pigs transported by road for 4 h.

MATERIALS AND METHODS

Experimental site and meteorological conditions

The experiment was performed at the faculty of veterinary medicine, Ahmadu Bello, university, Samaru, Zaria (11^0 $10^/$ N, 07^0 $38^/$ 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 consequently, transportation of pigs, especially during the season. During the study period, wet and dry-bulb temperatures were determined before transportation at the experimental site using dry and wet-bulb thermometers (Brannan, England) and rela-tive humidity (RH) was calculated using the manufacturer's standard manual attached. The dry-bulb temperatures and RH were also recorded at 06:00, 13:00 and 18:00 h for three consecutive days post-transportation.

Experimental animals and management

29 local pigs served as subjects. They comprised males and non-pregnant, non-nursing females of different age groups, ranging from 9 to 12 months and bought 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 m x 2.55 m with half the length to the roof without block work, which provided adequate ventilation. The pigs were not restrained inside the pen. They were kept under an intensive system of management. The pigs were pre-conditioned for 2 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 were treated using oxytetracycline (Kepro B. V®, Hollland) deep intramuscular at the dose of 20 mg/kg and thiabendazole ((M.S.D AGVET®, U.S.A.) at the dose rate of 25 mg/kg, respectively.

Experimental design, transportation of animals and blood sample collection

On the experimental day, 16 pigs were orally and individually administered ascorbic acid (Juhel® Nigeria Ltd., Enugu, Nigeria) at 250 mg/kg (Chervyakov et al., 1977) dissolved in 20 ml of water, while 13 pigs which served as control were given 20 ml of sterile water. These administrations were made immediately before loading the pigs into the vehicle. Food and water were withdrawn 12 h before the journey and throughout the journey period. The vehicle travelled from faculty of veterinary medicine, Ahmadu Bello university, Zaria on tarred road along Zaria-Jos road, covering a total distance of 140 km at a speed range of 40-50 km/h. The journey took 4 h (short-term duration), including stop-overs for police checkings. After completing the journey, the pigs were unloaded at the original loading point, fed and watered as they had been prior to the journey.

Blood samples were taken early in the morning a day before transportation, immediately and a week after transportation. 5 mm of blood was drawn aseptically via the anterior vena cava using a 10 ml syringe and 18 gauge x $1^1/2$ inch sterile needle from each animal. The blood was immediately poured inside a sample bottle, 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 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 concentration. 1 ml pipette was used to transfer exactly 0.02 ml of blood sample into each of the 10 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^{o}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 centrifuged at 1,500 x g for 15 min. The supernatant of each test tube

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

Hour	Ambient temperature (°C)			Relative humidity (%)	Wind speed (km/ day)
	Minimum	Maximum	Dry-bulb		
06: 00	13	24	14	24	226.43
13: 00	23	24	23	20	226.43
18: 00	21	22	21	19	
Mean ± S. E. M	19.00 ± 3.1	23.33 ± 0.7	19.33 ± 2.7	21.00 ± 0.51	

Table 2. Meteorological data from the study period post-transportation.

Hour	Ambient temperature (°C)			Relative humidity (%)	Wind speed (km/ day)
	Minimum	Maximum	Dry-bulb		
06: 00	15	25	14	26	259.51
13: 00	24	26	24	20	259.51
18: 00	23	21	20	20	
Mean ± S. E. M	20.67 ± 2.85	24.00 ± 1.53	19.33 ± 2.91	21.00 ± 0.67	

was transferred into a glass cuvette. The concentration of haemoglobin in the supernatant solution was measured using a spectrophotometer (Spectronic-20, Philip Harris Limited, Shenstone, UK) at 540 nm 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).

Haemolysis (%) = (OD of test/OD of distilled water) x 100

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 are shown in Tables 1 and 2. The period was characterized by relatively low values of minimum ambient temperature of 19.0 ± 3.1°C and low maximum ambient temperature of 23.3 ± 0.7°C. The dry-bulb temperatures value obtained during the recordings was 19.3 ± 2.7°C. The harmattan season was characterized by relatively low humidity of 21.00 ± 0.51%. The wind direction was North-east, and the speed was 226.43 km/ day.

The meteorological data during the post-transportation period (Table 2) were similar to those obtained during the pre-transportation period ($P > 0.05$) (Tables 1 and 2).

Effect of ascorbic acid administration on erythrocyte osmotic fragility of pigs transported by road for 4 h

The minimum and maximum haemolysis of erythrocytes occurred at 0.85 and 0.20% in the experimental pigs, while the corresponding values in the control pigs were at 0.85 and 0.50%, respectively, a day before the journey. All the values recorded at different concentrations before road transportation in experimental and control pigs (Figure 1) were not significantly ($P < 0.05$) different. Immediately after the journey, the minimum and maximum values of haemolysis were obtained at 0.85 and 0.50%, respectively in the control pigs; while in the experimental pigs, the corresponding values were obtained at 0.85 and 0.30%, respectively. At NaCl concentrations of 0.70, 0.80 and 0.85%, significant ($P < 0.05$) differences existed between the values recorded in experimental and control pigs (Figure 2). However, on day 7 after transportation, significance difference ($P < 0.05$) existed at the concentration of 0.40 and 0.50% between the experimental and control pigs (Figure 3), with the minimum value occurring at 0.85% in both experimental and control pigs and the maximum haemolysis occurred at 0.1 and 0.50% in the experimental and control pigs, respectively.

DISCUSSION

The results obtained in the present study demonstrated that the transported pigs were subjected to a cold and

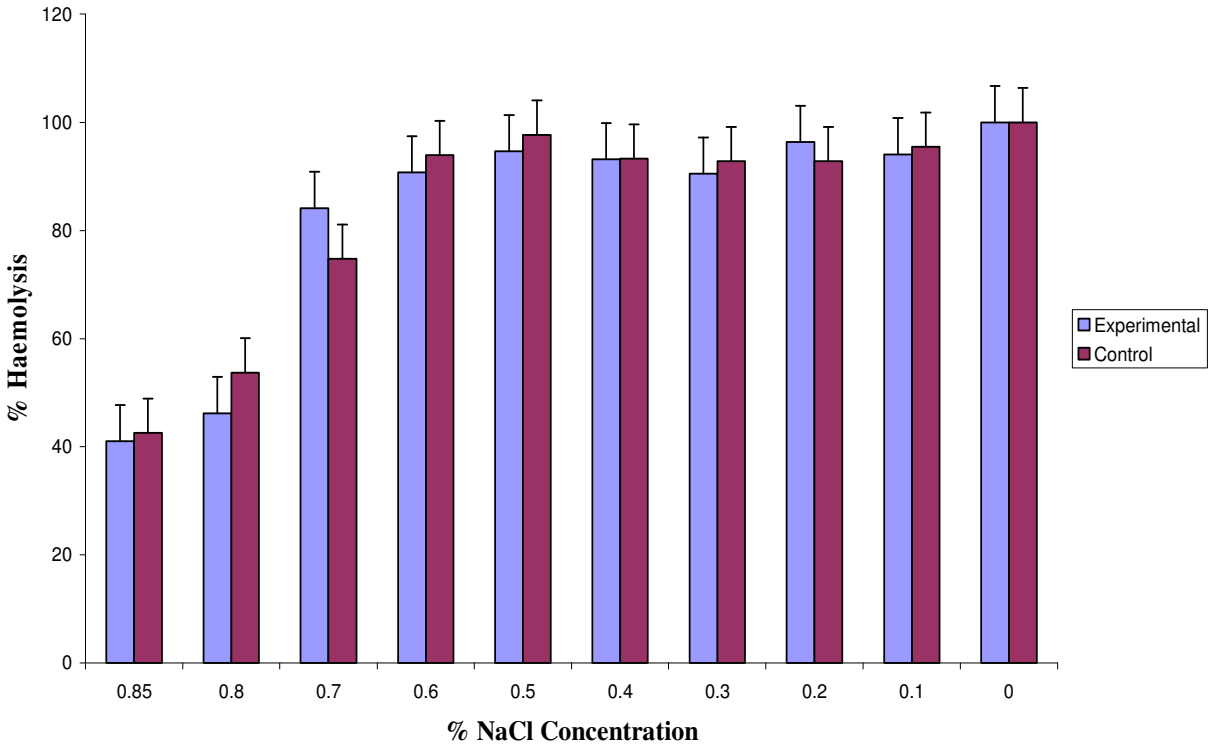

Figure 1. Effect of ascorbic acid on erythrocytes osmotic fragility before 4 h of road transportation in pigs.

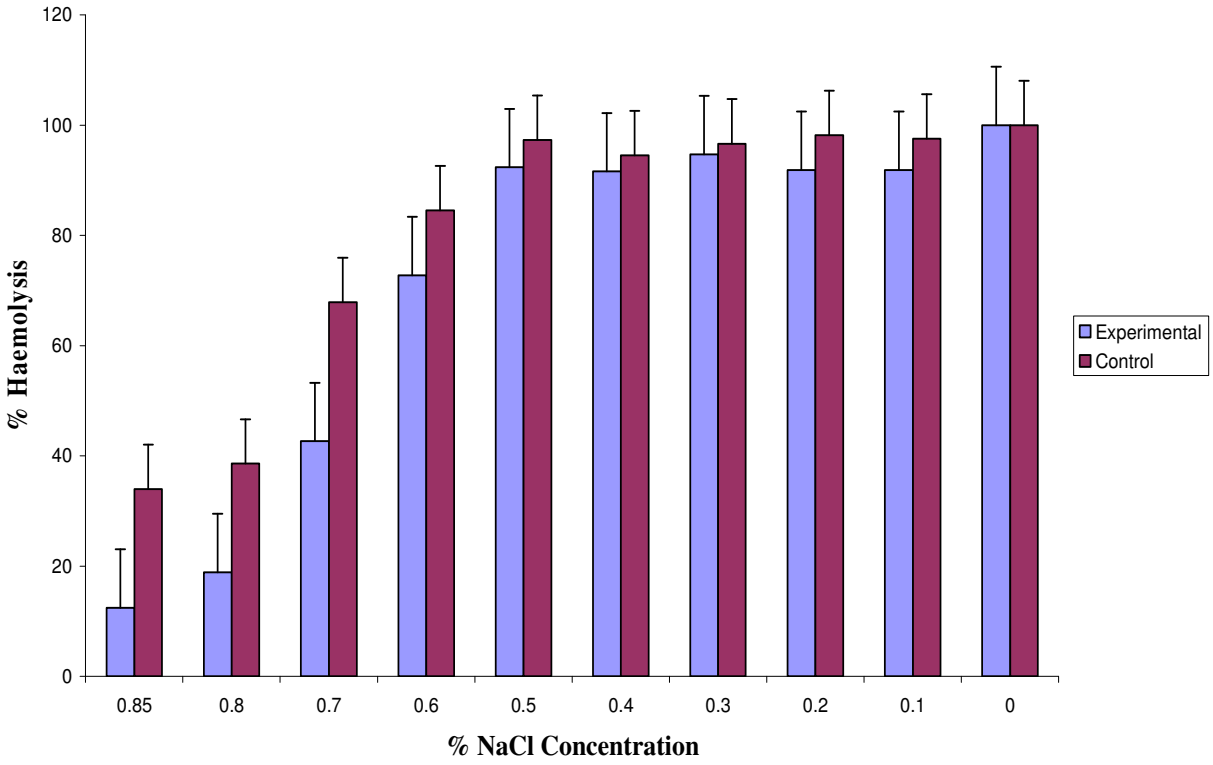

Figure 2. Effect of ascorbic acid on erythrocytes osmotic fragility immediately (within 30 min) 4 h of road transportation in pigs.

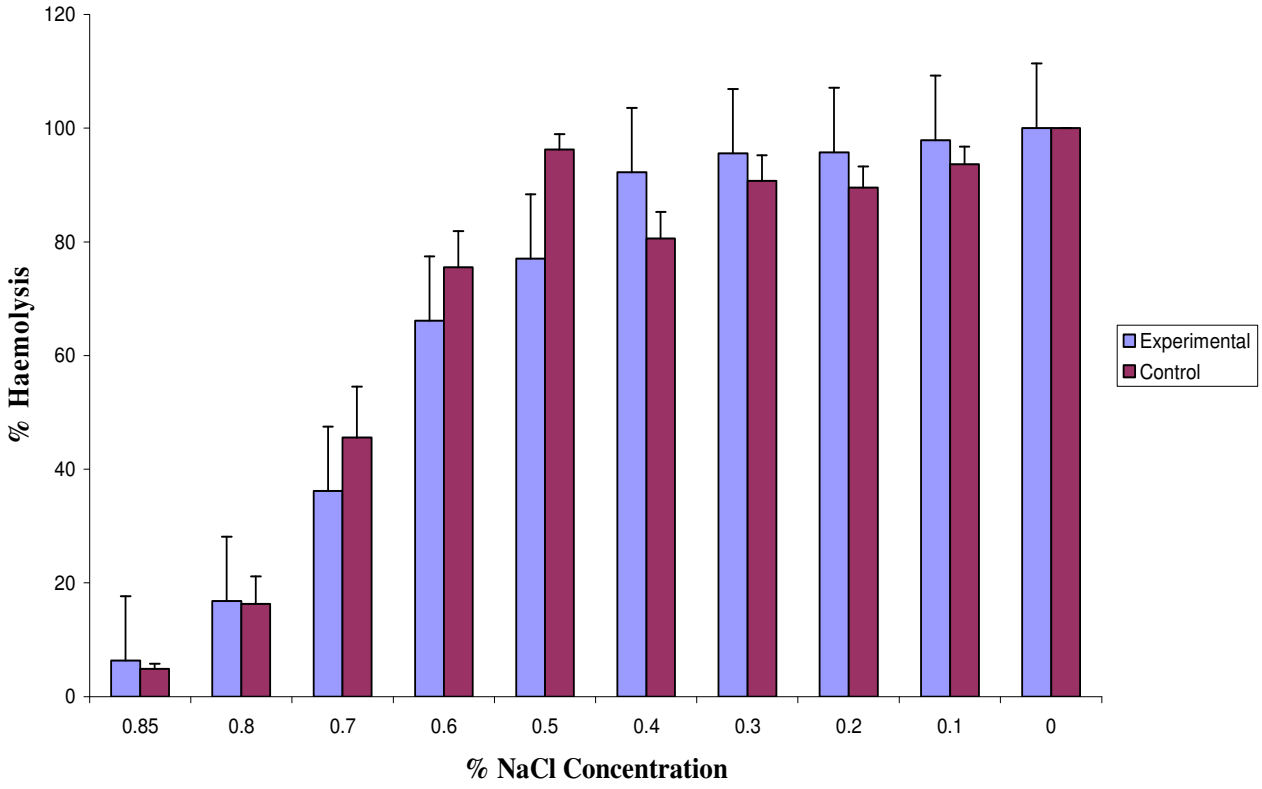

Figure 3. Effect of ascorbic acid on erythrocytes osmotic fragility on day 7 after 4 h of road transportation in pigs.

dust-laden wind with low ambient temperature, classical of the harmattan season in the northern guinea savannah zone of Nigeria (Ayo et al., 1998a,b). Meteorological results obtained during the present study agree with the previous findings that the harmattan season is thermally stressful to pigs (Adenkola and Ayo, 2006; Adenkola et al., 2007).

The erythrocyte osmotic fragility in hypotonic solution has been studied in various mammals (Olusanya and Adepoju, 1979; Oyewale, 1992) and it has been shown that it is related to geometric configuration of the erythrocytes, which in turn depends on the integrity of the cell membrane (Schalm et al., 1975). Before transportation, there was no significant difference between erythrocyte osmotic fragility of experimental and control pigs, but after the transportation the erythrocyte osmotic fragility was significantly different between the 2 groups. The results showed, for the first time, that erythrocyte osmotic fragility test may serve as an indicator of road transportation stress in pigs and that it is of diagnostic value in stress due to road transportation of pigs during the harmattan season.

The maximum erythrocyte osmotic fragility obtained in this study disagreed with that of Oladele et al. (2003), who reported maximum erythrocyte osmotic fragility at 0.15% NaCl concentration in chickens and guinea fowls during the harmattan season. The difference in the values may be attributed to species difference. The result of this study showed that road transportation stress, apparently, mediates its adverse effects via oxidant mechanisms. Thus immediately after transportation, haemolysis was higher in the control than experimental pigs. This finding was similar to those of Sumikawa et al. (1993) and Avellini et al. (1995) who observed that stress induced formation of free radicals which play a vital role in tissue damage and that they have deleterious effects on erythrocyte cytomembrane. Mossad et al. (2000) showed that haematological complications and autoimmne haemolytic anaemia observed in malignant lymphomas were attributed to oxidative stress. Thus, the results of the present study indicated that road transportation-induced stress enhances the generations of ROS, shown to increase haemolysis (Langsford and Zydney, 1993). Although free radicals were not measured directly in this study, it has been shown that they are generated in animals subjected to stress (Halliwell, 1996; Senturk et al., 2001; Chihuailaf et al., 2002). The observed increase in erythrocyte osmotic fragility in transported pigs further supports this fact. The higher haemolysis recor-ded in the control than experimental pigs post-transportation may be due to established impaired homeostatic mechanisms induced in animals not supplemented with ascorbic acid. The increase in ROS generation in the body, apparently, by road transportation stress on the body render-

ing cells more fragile and easily susceptible to hypotonic lysis. In contrast the lower percentage of haemolysis recorded in the experimental pigs was in agreement with observations of Senturk et al. (2001) and Candan et al. (2002) that ascorbic acid consolidates the integrity of erythrocyte membranes of and therefore reduces their oxidative damage. Oxidative stress occurs when the anti-oxidant defence systems in the body are overwhelmed by free radicals (Williams et al., 2008). Ascorbic acid administration to experimental pigs apparently, reduced the intensity of oxidant stress by enhancing the antioxidant defense mechanisms and suppressing the transportation stress which greatly minimized the destruction of erythrocyte. Therefore, an increase in osmotic fragility of erythrocytes, indicating a higher percentage of haemolysis obtained in control pigs may be due to exertional oxidant stress, prevented by ascorbic acid administration which increased the resistance of erythrocytes.

It has been established that ascorbic acid ameliorates the adverse effects of environmental stress (Tauler et al., 2003; Adenkola and Ayo, 2006; Minka and Ayo, 2007). Ascorbic acid has also been shown to prevent injurious effects of oxidants by the reduction of reactive oxygen and nitrogen species to stable molecules (Wilson, 2002). This fact may explain the finding in the present study that ascorbic acid administration in experimental pigs reduces erythrocyte osmotic fragility and the result is in agreement with that of Candan et al. (2002) and Chihuailaf et al. (2002) that ascorbic acid is a stress induced "quencher" capable of maintaining the integrity of the erythrocyte membrane.

The results obtained in the present study, for the first time demonstrated that the administration of ascorbic acid prior to transportation of pigs is beneficial because it reduces the accompanied stress associated with road transportation notable erythrocytes' fragility. It is, therefore, recommended that ascorbic acid be administered to pigs before transportation in order to reduce its adverse effects on pigs.

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