The Effects of Vitamins C and E on Erythrocyte Osmotic Fragility, Serum Malondialdehyde concentrations and Surface Erythrocyte Sialic Acid in Rams following Road Transportation

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ABSTRACT

This study was designed to investigate the effect of administration of combination of vitamin C + E and vitamin C alone on erythrocyte osmotic fragility (EOF), serum malondialdehyde (MDA) and surface sialic acid (SSA) concentration in road transported rams. Twenty one (21) rams were used for the study. On the day of transportation, 7 rams (Group 1) were orally and individually administered with vitamin C (Juhel® Nigeria Ltd.) at the dose of 250 mg/kg dissolved in 10 ml of water and also vitamin E (100 mg DL-α-tocopherol) (Patterson Zoochonist Ltd. Nigeria) at the dose of 75 mg/kg, per os while another 7 rams (Group 2) was administered orally and individually with vitamin C (250 mg/kg) only. The 3rd group (7 rams) was the control, and they were administered orally and individually with only 10 ml of sterile water. The results indicated that the percentage haemolysis after 8 hours (duration of the journey) increased in the entire treatment group which was significantly (P < 0.05) higher in the control group. On day 3 post-transportation, the obtained value was significantly (P < 0.05) lower in the antioxidant treated group. The obtained MDA value of 2.13 ± 0.51 µmol in the first hour of the journey and 2.43 ± 0.22 µmol in the 8th hour was significantly (P < 0.05) higher in the group 3 (control) than the values obtained in the treated groups. The values rose slightly in all groups 3-days post-transportation above pre-transportation value which was not significantly (P > 0.05) different. There was progressive decline in the mean SSA concentrations in the control group with a value of 6.00 ± mg/ml at the end of the journey (8 h) which was not significantly (P > 0.05) different but had higher decrease of 17.43 % as against 4.52 % and 5.56 % in group 1 and 2 respectively. In conclusion the findings in this study have provided an insight into the role of erythrocyte SSA and ROS in explaining haemolysis associated with road transported ram and the possible ameliorative roles of Vitamins C and E on haemolysis.

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1. INTRODUCTION

Livestock transportation by train is not common in Nigeria, because animals must first be transported to railway station and reloaded, and is restricted to certain parts of the country while the price of aircraft transportation limits its use to day-old chicks and transportation by ships is mostly used in roll-off situations (Adenkola, 2010). Thus, road transportation is the commonest means of transporting all livestock species in many countries of the world (Vecerek et al., 2006; Buckam-Sporer et al., 2008), including Nigeria (Adenkola and Ayo, 2009a), as it involves a series of handling and confinement situations which are unavoidably stressful (Hartung, 2003; Gupta et al., 2007) as a result of generation of reactive oxygen species (ROS) which leads to oxidative stress (Adenkola, 2010, EL-Gazzar, 2014). In order to reduce the adverse effects of road transportation on food animals and the economic losses encountered during animal transportation, developed countries and the entire EU legislators worked out Welfare of Animal Transport Order 1997. The order allows a maximum journey time of 19 h that must include a one-hour break for food, water and rest after each 8-9 h. This standard is currently undergoing a review again for improved welfare order (Gavinelli and Simonin, 2003).
One of the remarkable features of road transported animals is an increase in erythrocyte osmotic fragility, malondialdehyde concentration (Adenkola and Ayo, 2009a; Adenkola et al., 2010). Stress or physical exercise can modify an animal’s physiological mechanism (Hassan et al., 2015). The ameliorating effects of the vitamins are well manifested when the body ascorbic acid and or alpha-tocopherol is either overwhelmed or exhausted as a result of many stress factors that overtax the animals control system (Mckee and Harrison). Thus supplementation of the animal by exogenous antioxidants such as alpha-tocopherol and ascorbic acid (Niki, 2010) improves the antioxidant status, and seems to be very helpful to fight free radicals, because exposure of animal to stress has been demonstrated to induce an increase in free radicals in the body (Powers and Jackson, 2008). The role of erythrocyte membrane sialic acids during aging has been established however the relationship between sialic acid and oxidative stress is not clearly understood (Mohammed et al., 2012). This present work was done to investigate the effect of oxidative stress on erythrocyte membrane sialic acid, erythrocyte osmotic fragility and serum malondialdehyde concentration in rams and the possible ameliorative roles of vitamins C and E.

2. Materials and methods

2.1 Experimental Site and Meteorological Conditions

The study was conducted at Small Ruminant Unit of the University of Agriculture Teaching and Research Farm Makurdi (07°41’N, 08°37’E) in the Southern Guinea Savannah Zone of Nigeria. The area has a very warm weather with daily mean temperature ranging from 26.5 – 42.00°C, with an annual rainfall of 1,317 – 1,323 mm which span between 6 – 7 months (Adenkola et al., 2010). The meteorological data for the study period which included the minimum and maximum ambient temperature (AT), relative humidity (RH), rainfall, sunshine hour per day, wind speed and wind direction for the period of study was obtained from the Nigerian meteorological Agency (NIMET), Makurdi.

2.1 Experimental Design

On each experimental day, 7 rams (Group 1) were orally and individually administered with Vitamin C (VC) (Juhe® Nigeria Ltd.) at the dose of 250 mg/kg (Chervyakov et al., 1977) dissolved in 10 ml of water and also Vitamin E (VE) (100 mg DL-α-tocopherol) (Patterson Zoochonist Ltd. Nigeria) at the dose of 75 mg/kg, while another 7 rams (Group 2) was administered orally and individually with VC (250 mg/kg) only. The 3rd group (7 rams) was the control, and they were administered orally and individually with only 10 ml of sterile water. The administrations were made immediately before loading the rams into the vehicle. A standard Peugeot bus (35), was used to transport the rams. The inner compartment of the vehicle measured 3.63 x 1.35 x 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 0.71 m 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. The journey commenced at 8:00 am.

Food and water were withdrawn 12 hours before and throughout the journey period, which lasted 8 hours. For the journey duration, the vehicle travelled to and fro University of Agriculture, covering a total distance of 300 km, respectively. The speed of the vehicle was at a range of 40 - 50 km/h. After completing the journey, the rams were unloaded at the spot where they were originally loaded. The animals were given feed and water as they had been prior to the journey.

Blood samples were taken early in the morning a day before transportation, when no antioxidants were given to any of the rams, also 1 hr into the journey and immediately after transportation on arrival (8 hr) and 3 days after transportation. Five millimeters of blood was taken aseptically from the jugular vein using a 5 ml syringe and 18 gauge x 1 1/2 inch sterile needle from each ram. Two millimeters of blood for determination of haematological parameters was immediately poured inside a sample bottle, containing an anticoagulant, disodium salt of ethylene diaminetetra-acetic acid (EDTA) at the rate of 2 mg/ml of blood (Oyewale et al., 1992), while the remaining blood was immediately centrifuged 1,500 x g for 15 minutes and the resultant serum harvested, stored in the refrigerator until it is ready for use.

2.2 Evaluation of Serum Malondialdehyde Concentration, Erythrocyte Osmotic Fragility Determination and Assay for Erythrocytes Surface Sialic Acid.
Serum malonaldehyde (MDA) concentrations as a marker of lipid peroxidation was determined by the double heating method of Draper and Hadley (1990) as modified by Altuntas et al., (2002) while erythrocyte osmotic fragility (EOF) was determined using the method of Faulkner and King (1970) and free surface sialic acid and erythrocyte surface sialic acid values were determined using the procedures of Reuter and Schauer (1994).

2.3 Statistical Analysis
The data were subjected to two-way analysis of variance (ANOVA) and Pearson’s correlation analysis. The values were expressed as mean and standard error of mean (mean ± SEM). Values of P < 0.05 were considered significant.

3. Results
3.1 Meteorological Parameters during the Study Period
Meteorological Parameters
The minimum AT (20.35 ± 0.21°C) recorded was not significantly (P > 0.05) different from the maximum AT of 31.07 ± 0.21 obtained during the study period. The rainfall during the month of study was 12.38 ± 3.03 mm while the maximum relative humidity was high. The wind direction was mostly southerly while the wind was light breeze with an average speed of 2.00 ± 0.12 m/sec. (Table 1).

3.2 Variation in Malondialdehyde Concentration before, during and 3 Days after the Journey
The value obtained for serum MDA before transportation was not significantly different (P > 0.05) in all treatments before the journey. However 1 hour into the journey the value increased tremendously especially in the control group without any antioxidants with a value of 2.13 ± 0.51 µmol in the first hour of the journey and 2.43 ± 0.22 µmol in the 8th hour which was significantly (P < 0.05) higher than the values obtained in treatment 1 and 2. The values rose slightly in all groups 3-days post-transportation above pre-transportation value which was not significantly (P > 0.05) different.

3.3 Variation in Erythrocyte Osmotic Fragility before, during and 3 Days after the Journey
The fragiligram obtained in all the treatment pre-transportation was not significantly (P > 0.05) different at all the concentration of sodium chloride, however 1 h into the journey, a value of 46.4 ± % was recorded in the control group which was significantly (P < 0.05) higher than the corresponding values in treatment 1 & 2 with a value of 22.19 ± % and 27.17 ± % respectively. The percentage haemolysis in the 8 h (end of the journey) increased in the entire treatment group which was significantly (P < 0.05) higher in the control from 0.1 – 0.7 sodium chloride concentration, 3 days post-transportation value was significantly (P < 0.05) lower in the antioxidant treated group. A positive correlation (P < 0.001) existed between MDA concentration and erythrocyte osmotic fragility during the journey period.

3.4 Variation in Erythrocyte Bound Sialic before, during and 3 Days after the Journey
The obtained value of bound sialic acid in all the treatment groups were not significantly (P > 0.05) different before the journey, however a gradual progressive decline in the mean erythrocyte surface sialic acid concentrations was observed which was more in the control group with a value of 6.00 ± mg/ml at the end of the journey in the 8th hour which was not significantly (P > 0.05) different but had higher decrease of 17.43% as against 4.52% and 5.56% in group 1 and 2 respectively. There was a positive correlation between (P <0.001) MDA concentration and bound sialic acid during the journey period.

Table 1: Meteorological data during the Study Period ASFDHG

<table>
<thead>
<tr>
<th>Meteorological Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient Temperature Maximum (°C)</td>
<td>31.07 ± 0.21</td>
</tr>
<tr>
<td>Ambient Temperature Minimum (°C)</td>
<td>20.35 ± 0.21</td>
</tr>
<tr>
<td>Rainfall (mm)</td>
<td>12.38 ± 3.03</td>
</tr>
<tr>
<td>Relative Humidity High (%)</td>
<td>83.00 ± 0.00</td>
</tr>
<tr>
<td>Relative Humidity Low (%)</td>
<td>68.00 ± 0.00</td>
</tr>
<tr>
<td>Sunshine (hr/day)</td>
<td>6.62 ± 0.49</td>
</tr>
<tr>
<td>Wind Speed (m/sec)</td>
<td>2.00 ± 0.12</td>
</tr>
</tbody>
</table>
Figure 1: Fluctuation in Malondialdehyde Concentration before, during and 3 Days after the Journey

Figure 2: Effect of Vitamin C and E combination and Vitamin C alone on Erythrocyte Osmotic Fragility Pre-Transportation of Rams

Figure 3: Effect of Vitamin C and E combination and Vitamin C alone on Road Transported Rams Erythrocyte Osmotic Fragility 1-h into the Journey
Figure 4: Effect of Vitamin C and E combination and Vitamin C alone on Road Transported Rams Erythrocyte Osmotic Fragility immediately after the Journey (8-h)

Figure 5: Effect of Vitamin C and E combination and Vitamin C alone on Road Transported Rams Erythrocyte Osmotic Fragility 3 Days Post-Transportation

Figure 6: Effect of Vitamin C and E combination and Vitamin C alone on Road Transported Rams Bound Sialic acid Concentration.
4. Discussion

The meteorological parameters recorded during this period were in contrast with the findings of Adenkola et al. (2011) during harmattan and hot-dry season in the same locality. Rainy season has been described as the least stressful season of all the three seasons (harmattan, hot-dry and rainy seasons) in Nigeria (Igono et al., 1982; Oladele et al., 2003). The meteorological parameters obtained during the study period were within the established thermo neutral zone of for ruminant (Tarr, 2007) in the tropics and this condition did not have adverse effect on their homeostatic mechanism.

The non-significance in the degree of haemolysis obtained before transportation in all treatments group suggests that erythrocyte of these animals respond similarly to hypotonic solutions, before transportation. The integrity of red blood cell may be determined by measuring the changes in EOF and the normal function of the erythrocyte is largely hinged on the maintenance of the integrity of its membrane. EOF is used to determine the integrity of erythrocyte (Adenkola and Ayo, 2009a). Compromising this integrity result in increased erythrocyte fragility and the erythrocyte become more fragile thereby exposing them to destruction by macrophages (Lichtensteiger and Vinir, 2003) and hence greater haemolysis. Avellini et al. (1995) noted that ROS are known to play a vital role in tissue damage and has adverse effects on erythrocyte. It is known fact ROS are generated in animals subjected to stress (Halliwell, 1996) leading to lipid peroxidation a complex process which leads to membrane damage and cell death (Belge et al., 2003). Lipid peroxidation is the oxidative deterioration of polyunsaturated lipids, leading to the production of a degraded product called malondialdehyde (Belge et al., 2003) in which its concentration is a biomarker for ROS generation. The result of the present study showed that MDA concentration as well as the degree of haemolysis increases as the journey progresses especially in group 3 (control) which was not administered any antioxidant. Stress factors have been shown to cause increase generation of ROS and impair the activity of the antioxidant in vivo (Halliwell, 1996) and the depletion of these antioxidant systems could increase the vulnerability of tissues and of cellular components reactive oxidation species (Piccione et al., 2007). This finding support the earlier observation that increased MDA values can be due to stress (Belge et al., 2003) and that generation of MDA in vivo increases with exposure to environmental oxidants. Draper and Hadley (1990) assert that the generation of MDA in vivo increases with exposure to environmental oxidants. ROS attack on cell membranes results in formation of lipid peroxidation products such as MDA (Mates, 2000). The lipoperoxidative alteration in the structural and functional components of the erythrocyte membranes compromises their integrity, making it prone to increased erythrocyte fragility (Ambali et al., 2010) especially if there is imbalance in oxidant and antioxidant status in the body (Abou-Seif et al., 2000; Nazifi et al., 2009). The high polyunsaturated fatty acid content of the erythrocyte membrane and the continuous exposure to high concentrations of oxygen and iron (Kolanjiappan et al., 2002) in hemoglobin are factors that make erythrocytes very sensitive to oxidative injury (Kusmic et al., 2000).

Sialic acid, an acetylated derivative of neuroaminic acid (Erdogan et al., 2008) and it is attached to non reducing residues of carbohydrate chains of glycoproteins and glycolipids (Mohan and Priyav, 2010). Sialic acid has a variety of biological functions which includes binding and transport of positively charged molecules, as well as function as a protective shield for the terminal part of molecules or cells (Schauer and Kamerling, 1997). A dense layer of sialic acid molecules covers erythrocytes, and during their lifetime sialic acids were removed stepwise from the surface of the cells as they were ageing by the action of serum sialidase and by spontaneous hydrolysis and the unmasked erythrocytes are then bound to macrophages and phagocytosed (Bratosin et al., 1995). The decrease in bound sialic acid seen in control group could be due to ROS generated as indicated by high concentration of MDA. The obtained results which suggests a progressive haemolysis as the journey progresses is an indication that road transportation of livestock is really stressful. The decrease in blood pH and increase in lactate and peroxide caused by anaerobic exercise promote erythrocyte osmotic fragility as well as increased ROS and reduced surface sialic acid. In an earlier study (Adenkola and Ayo, 2009; Adenkola et al., 2010) in which pigs were transported for long and short term duration respectively after administration of ascorbic acid and the obtained results indicated that ascorbic acid protected the integrity of the erythrocyte membrane in experimental pigs administered ascorbic acid following road transportation as demonstrated by low haemolysis immediately after road transportation.
Therefore the finding that reduced surface erythrocyte surface sialic acid occurred concurrently with increased ROS concentration and increased haemolysis in control group (group 3) suggest that ROS might be cleaving off erythrocyte surface sialic acid, thus rendering them prone to phagocytosis. Tkaczyk and Vizek (2007) reported that ROS play a vital role in cellular and tissue damage and it has also been demonstrated to have adverse effects on erythrocytes (Sumikawa et al., 1993; Avellini et al., 1995). It is thus postulated that haemolysis seen in this study as a result of road transportation stress could possibly be due to increase production of ROS which cleave off surface sialic acid from the erythrocyte thereby making them prone to phagocytosis and lysis. However in group 1 and 2, in which VC + VE and VC alone was given was better, because the antioxidants possibly protected the membrane integrity and prevented ROS from cleaving off the surface erythrocyte or possibly the amount of ROS generated is not enough to cleave off the surface sialic acid in the presence of the administered antioxidants.

Ascorbic acid is a potent antioxidant compound used to mitigate adverse effects of stress in livestock (Adenkola and Ayo, 2009b) and is depleted from the body in stress situations (Hesta et al., 2008). As an antioxidant, it plays a fundamental role in the antioxidant defense, both as a scavenger and possibly in the regeneration of other antioxidants (Urban-Chmeil et al., 2009). It has the ability to sequester the singlet oxygen radical, stabilize the hydroxyl radical, and regenerate reduced vitamin E back to its active state, which functions to end peroxidation of cellular lipid membranes (Harrison and May, 2009). VE inhibits the effects of hydrogen protons and free radicals by saturating them, and so inhibits autooxidation (McDowell, 1989). VC has been demonstrated to enhance antioxidant activity of VE by reducing the tocopheroxyl radicals back to their active form of VE (Wei et al., 2005) or by sparing available VE (Retsky and Frei, 1995).

In conclusion the findings in this study have provided an insight into the role of erythrocyte surface sialic acid and ROS in explaining haemolysis associated with road transported ram and the possible role of antioxidant in ameliorating haemolysis and oxidative stress in antioxidant treated groups.

5. References


