Nigerian Veterinary Journal
Vol 35 (2) 995-1006
ARTICLE

Serum Malondialdehyde Concentration, Rectal Temperature and Excitability Score in Road Transported Rams Administered with Vitamins C + E Combination and Vitamin C

ADENKOLA, A.Y.* and OKORO, L.I.

Department of Physiology Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria. *Corresponding author: E-mail: aadenkola@yahoo.com, Tel +2348054977696

SUMMARY

Experiment was done to investigate the effect of administration of vitamin C + E and vitamin C alone on serum malondialdehyde concentration, rectal temperature and excitability score in road transported rams. On experimental day, 7 rams (Group 1) were administered with vitamin C at the dose of 250 mg/kg dissolved in 10 ml of water and also vitamin E at the dose of 75 mg/kg, per os while another 7 rams (Group 2) was administered with vitamin C (250 mg/kg) only. The 3rd groups (7 rams), the control, were administered with 10 ml of sterile water. The RT was taken at 07:00, 13:00 and 18:00 h, a total of five times, twice in a week and during the journey, at 1 h, 4 h and 8 h, and also three consecutive day's post-transportation. Blood samples were taken a day before transportation, immediately after, on arrival and a 3 days after transportation in which the serum was harvested for MDA determination. Excitability scores was recorded before loading into the vehicle, and immediately after unloading. The results indicated that the rectal temperature (RT) value was lowest at 07:00 hr in the Group 1, and even maintained 13:00 hr which was significantly ($P < 0.05$) different. The RT value fluctuates during the journey however this values in group 1 and group 2 was not significantly ($P > 0.05$) different. The serum malondialdehyde concentration value rose in the first hour (2.13 ± 0.51 ng/ml) of the journey to 2.43 ± 0.22 ng/ml in the 8th hour in control group, which was significantly ($P < 0.05$) higher than the values in the other groups.

In conclusion, road transportation of ram is stressful but this was reduced 3-days post-transportation and also administration of antioxidants vitamins VE +VC, and VC, alleviated adverse effect of road transportation stress in rams.

Keyword: Serum malondialdehyde, rectal temperature, excitability score, road transported ram, vitamin C, vitamin E.

INTRODUCTION

Road transportation of livestock is a critical phase in animal production and utilization and often considered as one of the main causes of stress raising considerable interest, both in economic and animal welfare terms (MORMEDE et al., 1982). Transportation of small ruminants' sheep and goat is fast expanding worldwide due to demand for their meat and skin used in leather work, and most of the studies done were in the temperate regions of the world (KANNAN et al., 2002) which has contradicting climatic conditions to the tropical world. During road transportation, animals are exposed to a variety of potential stressors such as motion of the vehicle, poor road condition, noise, vibrations, poor air quality, and lack of water and feed (BROOM, 2000, HARTUNG, 2003). The combined effect of these stressor have been reported to impair the homeostatic control mechanism of the animal; thus resulting in metabolic shift and altered bodily physiological measurements (BARRIO et al., 1993) and stress to animals. This stress is directly related to lipid peroxidation (McCLELLAND, 2004) and reactive oxygen species (ROS) which are produced faster than they can be safely neutralized by antioxidant mechanism hence oxidative stress results (TREVISAN et al., 2001). An imbalance between the generation and neutralization of ROS due to altered redox homoestasis within the cell leads to oxidative stress (TREVISAN et al., 2001; POWERS and JACKSON 2008,). This imbalance may be either due to an overproduction of ROS or deficiency of the antioxidant system and this oxidative stress is highly deleterious to cell as it causes damage to cell membrane (HALLIWELL, 2000). Stress factors have been demonstrated to impair the activity of antioxidant in vivo (SAHIN et al., 2001).

Livestock and human body system is equipped with a complete armory of defenses against external and internal aggressions such as ROS (SIES, 1993, SHARMA et al., 2009). The concentrations of ROS are kept under strict control by the activity of a complex defense system including enzyme and nonenzymatic species such as ascorbic acid or vitamin C (VC) and vitamin E (VE) (KOVACIC and SOMANATHAN, (2008). VC scavenges ROS in the aqueous part of the cell, whereas VE is the major scavenger of ROS in the lipid phase of cell membrane (HALLIWELL, 2006, NAZIROGLU, 2007

Therefore the aim of this present work is to determine the ameliorative effect of co-administration of VC and VE and singly administration of VC on serum malondialdehyde concentration on rectal temperature (RT) and excitability score (ES) in ram transported for 8 hours. Serum malondialdeyde (MDA) concentration was measured because MDA is one of the end-products of lipid peroxidation (LATA et al., 2004).

MATERIALS and METHODS

Experimental site and meteorological conditions

The study was conducted at Small Ruminant Unit of the University of Agriculture, Teaching and Research Farm Makurdi (070 41/ N, 080 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).

Experimental design

On each experimental day, 7 rams (Group 1) were orally and individually administered with VC (JUHEL® NIGERIA LTD.) at the dose of 250 mg/kg (CHERVYAKOV et al., 1977) dissolved in 10 ml of water and also VE (100 mg DL-α-tocopherol) (PATTERSON ZOOCHNIST LTD. NIGERIA) at the dose of 75 mg/kg, per os 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. Prior to transportation the RT were taken at 07:00, 13:00 and 18:00 h for a total of five times, twice in a week, during the journey, at 1 h, 4 h and 8 h, and also for three consecutive day's post-transportation. On the day of transportation, the rams were treated (all the groups) as done earlier before the journey. The administrations were made immediately before loading the ram into the vehicle.

Vehicle design, loading, and the journey

A standard Peugeot bus (J5), popularly used in the middle belt region of Nigeria for transportation of livestock 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 0.71m 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 8:00 am on the day of transportation.

Food and water were withdrawn 12 hours before and throughout the journey period, which lasted 8 hours. 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. The journey duration included stop-overs, to measure RT, very briefly (about 20 minutes) and for police-checking. 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, immediately after transportation, on arrival and a 3 days after transportation. Two millimeters of blood was taken aseptically from the jugular vein using a 2 ml syringe and 18 gauge x 11/2 inch sterile needle from each animal without an anticoagulant. The sample was immediately transferred to Physiology laboratory, University of Agriculture, Makurdi where the blood was immediately centrifuged 1,500 x g for 15 minutes and the resultant serum harvested for MDA determination.

Evaluation of serum malonaldehyde concentration

Serum malonaldehyde (MDA) concentration a marker of lipid peroxidation was determined by the double heating method of DRAPER and HADLEY (1990) as modified by ALTUNTAS et al., (2002). The principle of the method was spectrophotometric measurement of the colour produced during the reaction of thiobarbituric acid (TBA) with MDA. Briefly, 2.5 ml of 100 g/L of trichloroacetic acid was added to 0.5 ml of serum in a centrifuge tube and placed in a boiling water bath for 15 min. After cooling in tap water, the mixture was centrifuged at 1000 x g for 10 min, and 2ml of the supernatant was added to 1 ml of 6.7 g/L TBA solution in a test tube and placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance measured using a UV spectrophotometer (JENWAY, 6405 MODEL, JAPAN) at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex (1.56 x 10 5 cm-1), and expressed in μmol/ml

Measurement of rectal temperature

The RT was measured using a standard clinical thermometer (HARTMAN

DIGITAL THERMOMETER®, GERMANY), inserted approximately 5 - 6 cm through the anus into the rectum of each ram (ZAYTSEV et al., 1971); and left until an alarm was heard, indicating the end of the reading usually after 30-40s. The rams were caught easily and restrained lightly for measurements, which were completed within 20 minutes.

Weighing procedure

The rams were weighed using a conventional weighing scale (PHILLIP HARRIS® COMPANY, ENGLAND). An individual whose weight was earlier recorded by the scale remained on the scale, while another person restrained the ram and handed it to the person on the weighing scale whose weight has already been determined. A reading on the scale of the person was subtracted from the second weight of the person and the ram to give the weight of each animal.

Measurement of excitability scores of transported rams

Excitability scores were recorded during weighing of each ram before loading into the vehicle, and immediately after unloading. They were measured as described by VOISINET et al. (1997) and MARIA et al. (2004). While weighing, a score of one to four was allocated to each ram by a single observer; a higher score representing a greater level of excitability. A score of one was allocated to a ram that was calm, and made little movement during the handling. Two was allocated to an animal that occasionally shook itself in an attempt to escape, while three was assigned to an animal that continuously attempted to free itself. A score of four was given to an animal that struggled violently throughout the entire weighing period.

Statistical analysis

Excitability score for each period of measurement were summed and results are presented as percentiles. Data were subjected to Analysis of Variance (ANOVA) Minitab version 16.00 and the data are expressed as Mean ± standard error of the mean (mean ± SEM). Values of $P < 0.05$ were considered significant.

RESULTS

Rectal temperature of the ram at the study site

The RT value was lowest at 07:00 hr in the Group 1 with a value of 38.57 ± 0.580 C, while that of the control (group 111) was 38.67 ± 0.580 C, the RT however rose to 39.05 ± 0.530 C in the group 111 animals while the value of 38.67 ± 0.530 C was maintained in the group 1 at 13:00 hr which was significantly ($P < 0.05$) different (Figure 1).

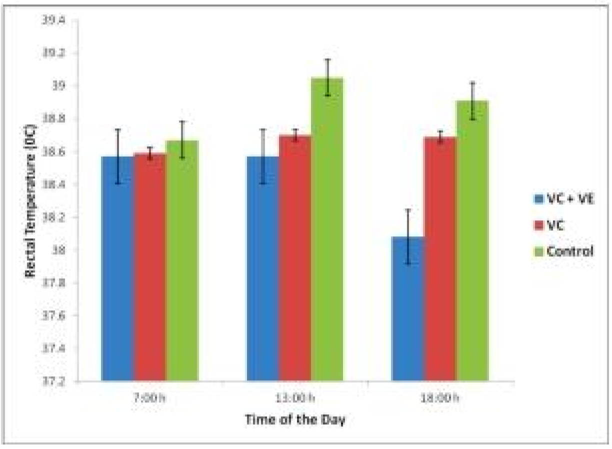

Figure 1: Rectal Temperature of the Rams at the Study Site

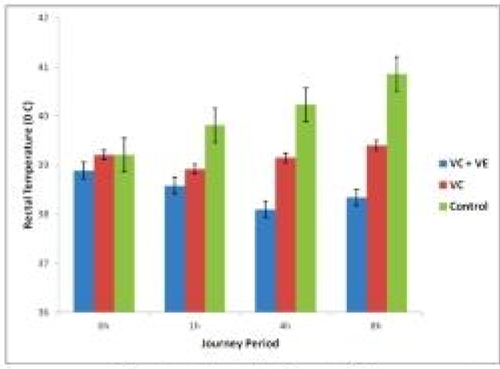

Figure 2: Fluctuation in Rectal Temperature of the Rams during the 8-h Road Transportation

Fluctuation in rectal temperature of the ram during the 8-h road transportation

The RT value obtained in group 3 shortly before the commencement of the journey was 39.21 ± 0.360 C and this value rose to 40.86 ± 0.340 C in the eighth hour of the journey ($P < 0.05$), RT value rose with duration of the journey, whereas the RT value decreased in group 1 from initial 38.89 ± 0.550 C before the journey to 38.34 ± 0.460 C in the eighth hour of the journey, while in the group 11 the value fluctuates from 39.21 ± 0.350 C at the beginning of the journey to 39.40 ± 0.280 C in the eighth hour, this values in group 1 and group 2 was not significantly ($P > 0.05$) different (Figure 2).

Rectal temperature of the ram 3-days post-transportation

The overall RT value 3 days after the journey in the group 3 was 38.25 ± 0.190 C at 07:00 h and rose to a value of 39.37± 0.290 C by 18:00 h, this values was not significantly ($P > 0.05$) different from the remaining treatments (Figure 3).

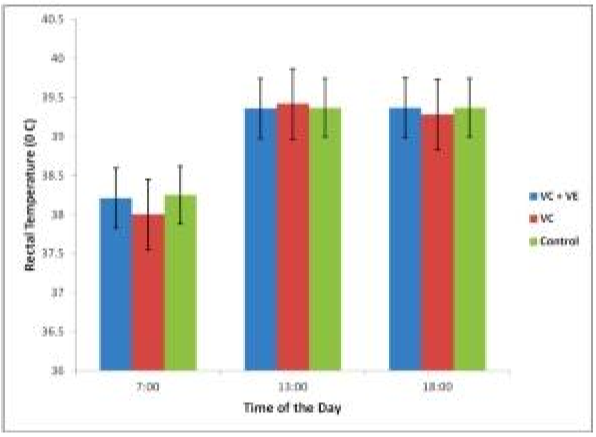

Figure 3: Rectal Temperature of the Ram 3-days Post-Transportation

Fluctuation in malondialdehyde concentration before, during and 3 days after the journey

The serum MDA concentration before transportation was 2.13 ± 0.43 µm/ml in group 3 while 1.95 ± 0.72 µm/ml and 1.85 ± 0.71 µm/ml was obtained in group 2 and 1 respectively which was not significantly ($P > 0.05$) different. The value rose from to 2.13 ± 0.51 µm/ml in the first hour of the journey and 2.43 ± 0.22 µm/ml in the 8th hour in the control group, this value was significantly ($P < 0.05$) higher than the obtained values in the other groups.

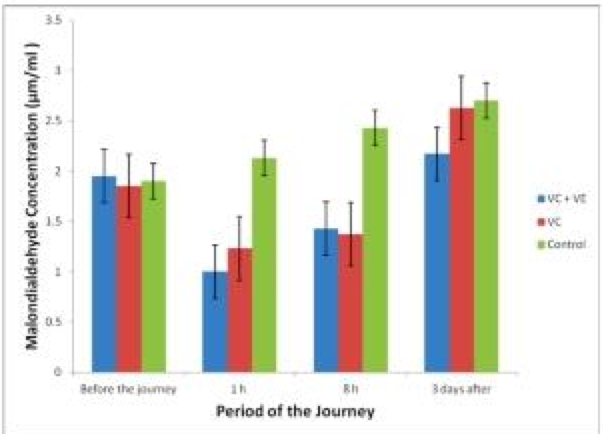

Figure 4: Fluctuation in Malondialdehyde Concentration before, during and 3 Days after the Journey

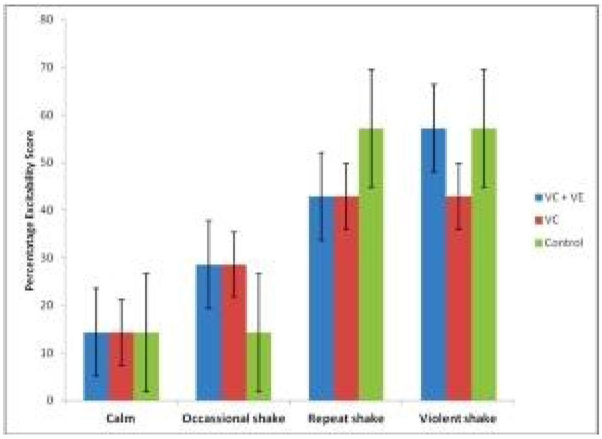

Figure 5: Excitability Score of Experimental and Control Rams before Transportation

Fluctuation in excitability score of experimental and control rams before, immediately after and 3 days after the journey

The results of the excitability scores in both experimental and control rams pre-transportation are shown in Figure 5. An excitability score of one was recorded in 14.29 ± 5.29 % in all the treatments before the journey, while a value of 57.14 ± 7.50 % and 42.86 ± 5.86 % was recorded in control, AA + E and AA respectively for an excitability score of four, which was not significantly ($P < 0.05$) different. Immediately after transportation, the highest excitability score of four was recorded with a value of 57.14 ± 7.50 % in the group 2, and the highest excitability score was obtained in group 2 also with a value of 85.71 ± 8.51 % while the lowest value of 14.29 was recorded in the control group (Figure 6). These value was significantly ($P < 0.05$) higher than the control group. However, there is no significant ($P < 0.05$) different in the obtained values for the scores 3 days post transportation (Figure 7).

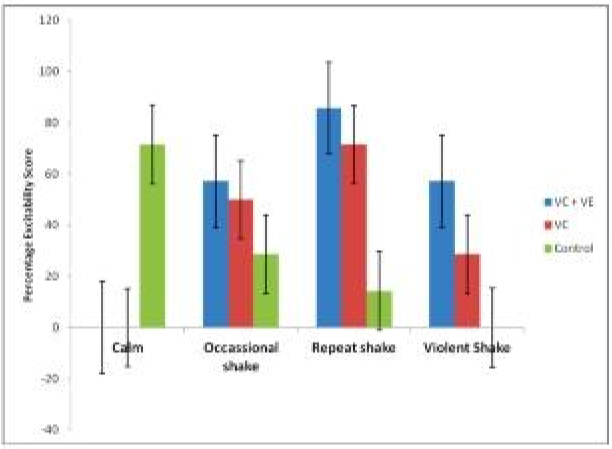

Figure 6: Excitability Score of Experimental and Control Rams After Transportation

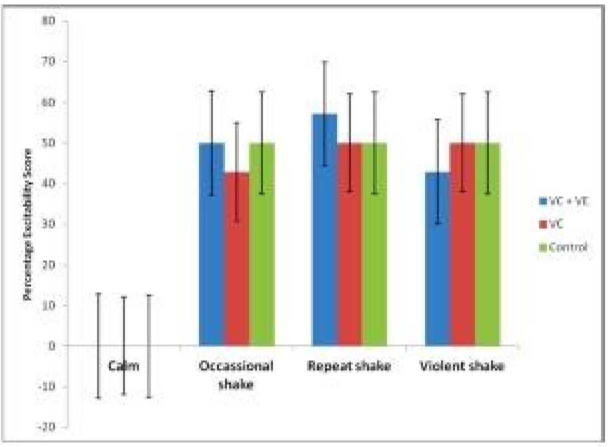

Figure 7: Excitability Score of Experimental and Control Rams 3 days Transportation

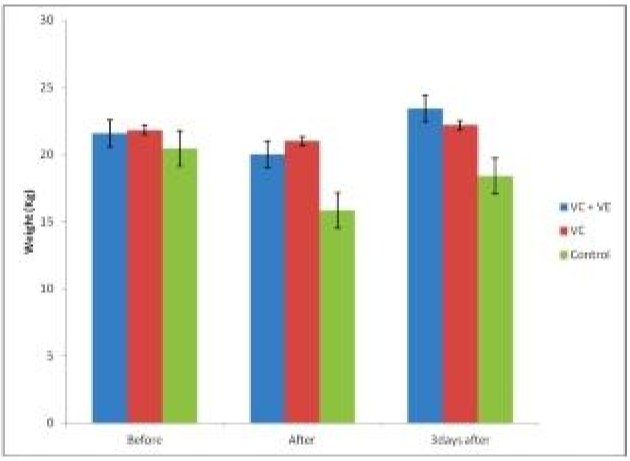

Figure 8: Fluctuation in Weight of Transported Rams, Before, After and 3 Days after the Journey

Fluctuation in weight of transported rams, before, after and 3 days after the journey The recorded weight of 20.43 ± 4.50 kg, 21.57 ± 2.50 kg and 21.83 ± 1.50 kg in group 1, 2 and 3 respectively was not significantly ($P > 0.05$) different before the journey, however, immediately after transportation a weight of 15.83 ± 0.85 kg which was significantly lower ($P < 0.05$) than the obtained values in the remaining 2 groups. Three days post-transportation, the values showed no significant (P > 0.05) different in the weight of the rams in all the groups.

DISCUSSION

The concentration of MDA has been widely applied as the most common biomarker for the assessment of lipid peroxidation in biological and medical sciences (SUTTNAR et al., 2001) because MDA is one of the end-products of lipid peroxidation, and the extent of lipid peroxidation is most frequently measured by estimating MDA levels (LATA et al., 2004). The higher concentration of MDA seen in this study during transportation especially in group 3 (control) which was not administered any antioxidant could be due to higher levels of glucocorticoids and adrenaline-induced pathways of aerobic energy production associated with stress, which generate reactive oxygen metabolites due to excessive oxidative damage and thus lipid peroxidation (FREEMAN and CRAPO, 1982; MOHAN and PRIYAV, 2010). These oxygen species in turn can oxidize many other important bio-molecules including membrane lipids (AKINWALE and ADEBULE, 2003).

However this process was modulated possibly by the antioxidant vitamins (VC + VE) which have been demonstrated to prevent or reduce considerably the ROS-induced damage to body cells (TAULER et al., 2003; PREGEL et al., 2005) because this ROS have been shown to induce lipoperoxidation of cytomembrane resulting in cell damage and destruction (ALTAN et al., 2003; SOLICHOVA et al., 2003). Also reduction of MDA in group 1 & 2 may be due to the enhancement of the fatty acid transport by antioxidants (VC &VE) into mitochondria for energy production, thereby lowering the availability of lipid for peroxidation. This result of this present study agrees with that of TOKARZEWSKI et al. (2004) who demonstrated a high level of MDA concentration in transported birds.

The RT values recorded were within the established normal range value documented for sheep in tropical environment (ADENKOLA et al., 2004). The results obtained indicate variations in RT of the rams at different hours of recordings as evidenced by gradual increase in RT from 06:00 to 18: 00 h in all the groups. The fact that the highest RT values recorded were at the hottest hour of the day (13:00 h) in group 3 animals agrees with the results of previous studies that RT fluctuates with ambient temperature in agreement with the established pattern of diurnal variations in RT values classical of most mammals. The classical fluctuations in RT values were in agreement with the findings of PICCIONE and CAOLA (2002) that RT values vary with the hour of the day and ambient temperature. The RT is a true reflection of internal body temperature and a reliable index of thermal balance (OZCAN et al., 2003; ZHAO et al., 2010). The result of this study demonstrated that body temperature was modulated in group 1 and 2 and that administration of VC and VE exerted hypothermia on the rams and that its administration attenuated the negative effect of the environmental stress apparently due to generation of free radicals that could have inhibited the hypothalamic thermostat (CHARKRABORTI et al., 2008) in this group of animals. Co- administration of VC and VE reduced the impact of meteorological stress and this reduction was stronger than when any of these vitamins was administered singly. This finding collaborates the with the observation of NOCKELS (1996) that chain breaking antioxidants such as lipid soluble vitamins are of value in controlling oxidants produced cells under environmental stress.

The RT values recorded in all the groups just before transportation fell within the established normal range of temperature documented for sheep (ADENKOLA et al., 2004). This indicated that the animals were healthy and, therefore, fit for the journey. This agrees with the findings of GRANDIN (2001) that physically fit animals should be transported. However, the RT values obtained 1 hour into the journey increased even to the 8th hour significantly ($P < 0.05$) and this effect is more in this group which was not administered any antioxidants. The effect seen could be attributed to the high concentration of free radicals generated as this free radical generation increases with the hour of journey and the effect is more in the group 3 in which an antioxidant was not administered, while the effect was less in the group in which the antioxidants was combined. Free radical generation has been known to inhibit the hypothalamic thermostat (CHARKRABORTI et al., 2008) in modulating the RT in animal subjected to environmental stress, and this environmental stress has been demonstrated to cause oxidative stress and impairs antioxidants in vivo (SAHIN et al., 2001) and therefore antioxidants supplementation 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). The finding in this study agrees with the earlier work of ADENKOLA et al. (2011) who administered VC, and transported pigs for eight hours. The modulating effects of the vitamins are well manifested when the body VC and VE is either overwhelmed or exhausted as a result of stress factors that overtax the animal control systems (MCKEE and HARRISON, 1995) and transported animals are often exposed to a variety of environmental extraneous stimuli, which are called stress factors (FRAZER and BROWN, 1990) and thus road transportation of livestock is very stressful to animals (CHANDRA and DAS, 2001; VOSLAROVA et al., 2007; ADENKOLA et al., 2011). Antioxidants vitamins have been shown to prevent or reduce considerably the free radicals-induced damages to body cells (TAULER et al., 2003). VE is a biological antioxidant soluble fat (HALLIWELL and GUTTERIDGE, 1991), which inhibits the oxidation of long chained fatty acids of the cell membrane (MCDOWELL, 1989; HENNKENS, 1986). Unsaturated fatty acids react with oxygen, and form superoxide and hydroperoxide (COLLACCHIO, 1989). These free radicals cause cell damage by disturbing the metabolism and structure of the biological membranes of those organs that contain excessive amount of unsaturated fatty acids (BIESALSKI, 1992). 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) and that VE is located in the membrane in such a way the phenolic hydroxyl group, an active site for radical scavenging, is placed at or near the surface and the phytyl side chain, is embedded like an anchor into interior of the membrane (TSUCHIHASHI et al., 1995).

The results obtained on the excitability scores demonstrated that transportation of rams, apparently, has adverse effects on the nervous system of the animals as evidenced by a decrease in the values of excitability scores in group 3. This progressive decrease in excitability score in the group 3 (control animals) reflected the state of physical and mental alertness of the animals, indicating sensorimotor reflex and neuromuscular coordination. This decrease may be due to generation of free radical which possibly induced lipoperoxidative damage to the brain (BROCARDO et al., 2007) and impairs the activity of the cerebral cortex, because brain is highly vulnerable to oxidative damage due to high utilization of inspired oxygen and the large amount of easily oxidized polyunsaturated fatty acid (BALU et al., 2005). Free radicals play an important role in neurodegenerative disorders by oxidizing the macromolecules like protein, deoxyribonucleic acid and lipids leading to the common final pathways for cell death (Harman, 1992; Sohal et al., 1995). However, group 1 and group 2 has an improved excitability score especially after road transportation, possibly because VC and VE has the ability to scavenge the enormous free radical generated and thereby aiding the restoration of neuromuscular function. VC has been demonstrated to be involved in the synthesis of neurotransmitter such as norepinephrine which may increase excitability score (BALZ, 2003). The result of the present study agrees with that obtained by ADENKOLA et al. (2009) who demonstrated the ability of ascorbic acid to mitigate low excitability score induced by road transportation stress in pigs.

There was a drastic reduction in the weight of all transported animals, possibly due to the fact the animals were not fed prior to transportation, and this may likely stimulate adrenocortical trophic hormone to produce glucocoticoids which causes glycogenolysis, this coupled with increase free radicals leads to oxidative stress and lipid peroxidation, all these may be involved in weight loss. The reverse is the case with those animals administered with antioxidants, possibly because the antioxidant prevent circulating glucocorticoid, and also because of their antioxidants activity by inhibiting free radical generation or scavenging the initiating free radical and terminating the radical propagation reaction.

In conclusion, road transportation of ram is stressful as indicated by increase in serum MDA, RT and ES; however this effect was reduced 3-days post-transportation and also administration of antioxidants vitamins VE + VC, and VC, alleviated adverse effect of road transportation stress in rams, co-administration of VE + VC in ram is not better than singly administration of VC alone.

REFERENCES

ADENKOLA, A.Y., KAANKUKA, F.G. and OYEDOKUN. T.T. (2004). Effect of Ascorbic Acid on Some Physiological and Haematological Parameters on West African Dwarf Sheep Confine during the Rainy Season. J Agric. Sci. Tech.14 (1&2), 8 – 12.

ADENKOLA, A.Y., AYO, J.O., SACKEY, A.K.B., ADELAIYE, A.B. and MINKA. N.S. (2009). Excitability scores of pigs administered ascorbic acid and transported during the harmattan season. Vet. Arhiv 79 (5), 471-480.

ADENKOLA, A.Y., KAANKUKA, F.G., IKYUME, T.T., ICHAVER., I.F. and YAAKUGH, I.D.I. (2010). Ascorbic acid effect on erythrocyte osmotic fragility, haematological parameters and performance of weaned rabbits at the end of rainy season in Makurdi, Nigeria. J. Anim. Plant Sci. 9(1), 1077 - 1085.

ADENKOLA, A.Y., AYO, J.O., SACKEY, A.K.B. and ADELAIYE. A.B. (2011). Ameliorative effect of ascorbic acid on rectal temperature of pigs transported by road for eight hours during the harmattan season. Agric. Biol. J. North Amer. 25, 734 – 741.

AKINWANDE, A.I. and ADEBULE, A.O.A. (2003). Ascorbic acid and beta-carotene alleviate oxidative effect of London Kingsize® cigarette smoke on tissue lipids. Nig. J. Hlth. Biochem. Sci. 2(1), 12 - 15.

ALTAN, O., PABACCUAOGLU, A., KONYALIOGLU, S. and BAYYRACTAR, H. (2003). Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. Bri. Poult. Sci. 44(4), 54 - 55.

ALTUNTAS, I., DELIBAS, N. and SUTCU, R. (2002). The effects of organophosphate insecticide methidathion on lipid peroxidation and anti-oxidation enzymes in rat erythrocyte: Role of vitamins E and C. Hum. Exp. Toxicol. 21, 681 – 685.

BARRIO, A.S., SCHRAMA, J.W., WANDEL, H.W., BETTMA, H.M. and VEDRSTEGEN. M.W.A. (1993). Energy metabolism of growing pigs after transportation, regrouping and exposure to new housing conditions as affected by feeding level. J. Anim. Sci. 71, 1754 - 1760.

BALU, M., SANGEETHA, P., HARIPIYA, D. and PANEERSELVAN. C. (2005). Rejuvenation of antiopxidants system in central nervous system of aged rats by grape extract. Neurosci. Letters 383, 295 – 300.

BALZ, F. (2003). Vitamin C intake. Nutri. Dis. 14, 1 - 18

BIESALSKI, H.K. (1992). Vitamins and carotenoid as biological antioxidants. Institute of Physiological Chemistry. University of Mainz 4/5, 3 – 10.

BROCARDO, P.S., ASSNI, F., FRANCO, J.L., PANDOLFO, P., MULLER, Y.M. and TAKAHASHI. R.N. (2007). Zinc attenuates malathion-induced depressant-like behavior and confers neuroprotection in the rat brain. Toxicol. Sci. 97, 140 - 48.

BROOM, D.M. (2000). Welfare assessment and welfare problem areas during handling and transport. In: GRANDIN, T. (Ed.). Livestock Handling and Transport, CABI Publishing, New York. Pp. 43 - 61.

CHARKRABORTI, A., GULATI, K. and RAY, A. (2008). Age-related differences in stress-induced neurobehaviour responses in rats: Modulation by antioxidants and nitrergic agents. Behav. Brain Res. 3, 186.

CHANDRAS, B.S. and DAS. N. (2001). The handling and short-haul road transportation of spent buffaloes in relation to bruising and animal welfare. Trop. Anim. Hlth Produc. 33, 155 - 163.

CHERVYAKOV, D.K., YEVDOKINOV, P.D. and VISHKER, A.S. (1977). Drugs in Veterinary Medicine. Kolos Publishing House, Moscow, 496pp.

COLLACHIO, T.A., MEMOLI, V.A. and HILDEBERT. L. (1989). Antioxidants. Arch. Surg. 124, 217 – 221.

DRAPER, H.H. and HADLEY. M. (1990). Malondialdehyde determination as index of lipid peroxidation, Methods Enzymol, 186, 421 - 431.

FRAZER, A.F. and BROWN. D.M. (1990). Farm Animal Behaviour and Welfare. 3rd Edition, CAB International, Wallingford, U.K.

FREEMAN, B.A. and Crapo. J.D. (1982). Biology of disease-free radicals and tissue injury. Lab Invest, 47, 412 – 426.

GRANDIN, T. (2001). Perspectives on transportation issues; the importance of having physically fit cattle and pigs. J. Anim. Sci. 79, 201 – 207.

HALLIWELL, B. (2000). The antioxidant Paradox, Lancet 355, 1179 – 1180.

HALLIWELL, B. (2006). Oxidative stress and neurodegeneration: Where are we now? J. Neurochem. 97, 1634 – 1658.

HARTUNG, J. (2003). Effects of transport on health of farm animals. Vet. Res. Comm. 27, 525 - 527.

KAFRI, I. and CHERRY. J.A. (1984). Supplemental ascorbic acid and heat stress in broiler chicks. Poul. Sci. 63(suppl.), 125

KANNAN, G., TERRILL, T.H., KONAKOU, B., GELAYE, S. and AMOAH. E.A. (2002). Simulated preslaughter holding and isolation effects on stress responses and live weight shrinkage in meat goats. J. Anim. Sci. 80, 1777 – 1780.

KLEIN, J.A. and ACKERMAN, S.L. (2003). Oxidative stress, cell cycle, and neurodegeneration. J. Clin. Invest. 111, 785 - 93.

KOVAIC, P. and SOMANATHAN. R. (2008). Unifying mechanism for eye toxicity: electron transfer, reactive oxygen species, antioxidant benefits, cell signaling and cell membranes. Cell Memb. Free Rad. Res. 2, 56 - 59.

LATA, H., AHUJA, G.K., NARANG, A.P.S. and WALIA. L. (2004). Effect of immobilization stress on lipid peroxidation and profile in rabbits. J. Clin. Biochem.19, 1 – 4.

MARIA, G.A., VILLAROEL, M., CHACON, G. and GEBRESENBET, G. (2004). Scoring system for evaluating the stress to cattle of commercial loading and unloading. The Vet. Rec. 154(26), 818 - 821.

MCCLELLAND, G.B. (2004). Fat to the fire: The regulation of lipid oxidation with exercise and environmental stress. J. Comp. Biochem. Physiol. 139, 44 – 460.

MCDOWELL, L.R. (1989). Vitamins in animals nutrition: VitaminC, folacin. In MCDOWELL, L. R. (Ed.), Comparative aspects to Human nutrition. Academic Press, London, UK. Pp298 – 387.

MCKEE, J.S. and HARRISON, P.C. (1995). Effects of supplemental ascorbic acid on the performance of broiler chickens exposed to multiple concurrent stressors. Poul. Sci. 74(11), 1772 – 1785.

MOHAN, S.K.. and PRIYAV, V. (2010). Serum total sialic acid, lipid peroxidation and glutathione reductase levels in patients with rheumatoid arthritis. Tur. J. Med. Sci. 40(4), 537 – 540.

MORMEDE, P., SOISSOUS, J., ESATHE, R.M., RAOULT, J., LEGARFF, G., LEVIEUX, D. and DANTZER. R. (1982). Effects of transportation on blood serum composition, disease incidence and production traits in young calves. Influence of the journey duration. Anim. Res. Vet. 13, 369 – 384.

NAZIROGLU, M. (2007). Molecular mechanisms of vitamin E on intracellular signaling pathways in the brain. In Laszlo Goth (Ed) Reactive Oxygen Species and Diseases. Research Signpost Press Kerala, India, pp 239 – 256.

NOCKELS, C.F. (1996). Antioxidants improve cattle immunity following stress. Anim. Feed Sci. Technol. 62, 59 – 68.

OZKAN, S.Y., AKBAS, O., ALTAN, A., ALTAN, V. and AYHAN. K. (2003). The effect of short-term fasting on performance traits and rectal temperature of broilers during the summer season. Bri. Poul. Sci. 44, 88 – 95.

PICCIONE, G. and CAOLA. G. (2002). Biological rhythm in livestock. J. Vet. Sci. 3, 145 – 157

PREGEL, P., BOLLO, E., CANNZO, F.T., BIOLATTI, B., CONTATO, E. and BIOLATTI. P.G. (2005). Antioxidants capacity as a reliable marker of stress in daily calves transported by road. Vet. Rec. 150, 53 – 54.

POWERS, S.K. and JACKSON, M.J. (2008). Exercise-induced oxidative stress: Cellular mechcanisms and impact on muscle force production. Physiolo. Rev.88, 1243 - 1276.

RETSKY, K.L. and FREI. B. (1985). Vitamin C prevents metal ion-dependent initiation and propagation of lipid peroxidation in human low-density lipoprotein. Biochem. Biophy. Acta, 1257: 279 – 287.

SAHIN, K., SAHIN, N., ONDERCI, M., YARALIOGU, S. and KUCUK, O.

(2001). Protective role of supplemental vitamin E on lipid peroxidation, vitamins E, A and some mineral concentrations of broilers reared under heat stress. Vet. Mex (Czechslosvakia) 46, 140 - 144.

SEN, C.K. (2001). Antioxidant in exercise. Nutr. Sports Med. 31, 891 – 908.

SHARMA, K.R., CHATTERJI, S., RAI, K.D., MEHTA, S.I., RAI, K.P., SINGH, K.R., WATAL, G. and SHARMA, B. (2009). Antioxidant activities and phenolic contents of the aqueous extracts of some Indian medicinal plants. J. Med. Plant Res. 3(11), 944 - 948.

SIES, H. (1993). Strategies of antioxidant defense. Eur. J. Biochem. 215, 213 - 219.

SOHAL, R.S., AGWARWAL, S. and SOHALI, B.H. (1995). Oxidative stress and aging in the Mongolian gerbil (Meriones unguiculatus). Mech. Ageing Develop. 81, 15 – 25.

SOLICHOVA, D., KORECKA, L., SVOBODOVA, I., MUSLI, F., BLAHA, V., ZDANSKY, P. and ZADAK, Z. (2003). Development and validation of HPLC method for the determination of α-tocopherol in human erythrocyte for clinical applications. Ann. Biann. Chem. 376, 444 – 447.

SULTNAR, J., MASOVA, L. and DYR, E. (2001). Influence of citrate and EDTA anticoagulants on plasma malondialdehyde concentrations estimated by high-performance. J. Chrom. Biol. B751, 193 – 197.

TAULER, P., AQUILO, A., GIMELO, I., FUENTASPINA, E., TUR, J.A. and PONS, A. (2003). Influence of vitamin C diet supplementation on endogenous antioxidant defences during exhaustive exercise. Eur. J. Physiol. 446, 658 - 664.

TREVISAN, M., BROWNE, R., RAM, MUTI, P., FRENDENHEIN, J., CAROSELLA, A.N. and ARMSTRONG, D. (2001). Correlates of makers of oxidative stress status in the general population. Amer. J. Epidemiol. 154, 348 – 356.

TOKARZEWSKI, S., WERNICKI, A., KANKOFER, M., and URBAN-CHIMELLI, R. (2004). Lipid peroxidation as the additional indicator of transport stress in broilers. Pol. J. Vet. Sci. 7(2), 109 – 112.

TSUCHIHASHI, H., KIGOSHI, M., IWATSUKI, M. and NIKI, E. (1995). Action of β- carotene as an antioxidant against lipid peroxidation. Acta Biochem. Biophy. 323, 137 – 147.

VOISNET, B.D., GRANDIN, T., O'CONNOR, S.F., TATUN, J.D. and DEESING, M.J. (1997). Bos indicus-cross feedlot cattle with excitable temperaments have tougher meat and higher incident of border-line dark cutters. Meat Sci. 46, 367-377.

VOSLAROVA, E., JANACKOVA, B., VITULA, F., KOZAK, A. and VECEREK, V. (2007). Effects of transport distance and the season of the year on death among hens and roosters in transport to poultry processing plants in Czech Republic in the period from 1997 to 2004. Vet. Med. 52(6), 262 - 266.

WARRIS, P.D. (1996). The welfare of animals during transport. The Vet. Ann. 36, 73 – 85.

WEI, M., AMOLD, L., CANO, M., and COHEN, S.M. (2005). Effects of co-administation of antioxidants and arsenicals on the rat urinary bladder epithelium. Toxicol. Sci. 83, 237 – 245.

ZHAO, Z., CAO, J., MENG, X. and LI, Y. (2010). Seasonal variation in metabolism and thermoregulation in the stripped hamster (Cricetulus barabensis). J. Thermal Biol. 35(1), 52 – 57.