



Effect of packing on changes in erythrocyte osmotic fragility and malondialdehyde concentration in donkeys administered with ascorbic acid

Authors:

Folashade Olaifa¹
Joseph O. Ayo¹
Suleiman F. Ambali¹
Peter I. Rekwot²

Affiliations:

¹Department of Physiology and Pharmacology, Ahmadu Bello University, Nigeria

²National Animal Production Research Institute, Ahmadu Bello University, Nigeria

Correspondence to:

Folashade Olaifa

Email:

folashadeakanmu@yahoo.com

Postal address:

Department of Physiology and Pharmacology, Ahmadu Bello University, Nigeria, 810000

Dates:

Received: 11 Feb. 2012
Accepted: 20 Aug. 2012
Published: 05 Dec. 2012

How to cite this article:

Olaifa, F., Ayo, J.O., Ambali, S.F. & Rekwot, P.I., 2012, 'Effect of packing on changes in erythrocyte osmotic fragility and malondialdehyde concentration in donkeys administered with ascorbic acid', *Onderstepoort Journal of Veterinary Research* 79(1), Art. #413, 5 pages.
<http://dx.doi.org/10.4102/ojvr.v79i1.413>

© 2012. The Authors.
Licensee: AOSIS OpenJournals. This work is licensed under the Creative Commons Attribution License.

Experiments were performed with the aim of investigating the effect of packing on erythrocyte osmotic fragility (EOF) and malondialdehyde (MDA) concentration in donkeys, and the effect of ascorbic acid (AA). Twelve apparently healthy donkeys raised under the traditional extensive system served as experimental subjects. Six donkeys administered orally with AA (200 mg/kg) and subjected to packing were used as experimental animals, whilst six others not administered with AA served as controls. Blood samples were collected pre- and post-packing from all the donkeys for the determination of MDA and EOF. At 0.3% Sodium Chloride (NaCl) concentration, the percentage haemolysis was $93.69\% \pm 2.21\%$ in the control donkeys and the value was significantly ($P < 0.05$) higher than the value of $71.31\% \pm 8.33\%$, recorded in the experimental donkeys. The post-packing MDA concentration obtained in the control donkeys was $39.62 \mu\text{mol} \pm 4.16 \mu\text{mol}$, and was not significantly different ($P > 0.05$) from the value of $35.97 \mu\text{mol} \pm 2.88 \mu\text{mol}$ recorded in the experimental donkeys. In conclusion, the increase in haemolysis obtained in the donkeys suggested that packing induced oxidative stress, which was ameliorated by AA administration.

Introduction

It has been established that erythrocyte osmotic fragility (EOF) is a good indicator of stress (Adenkola *et al.* 2010; Asala *et al.* 2011; Hesta *et al.* 2008). Changes in the EOF occurring during physical exertion such as in exercise in the equine are important indicators of intravascular haemolysis (Hanzawa & Watanabe 2000). Erythrocyte osmotic fragility has been used to evaluate stress due to transportation by road in pigs (Adenkola & Ayo 2009; Asala *et al.* 2011) and goats (Minka & Ayo 2010). The decrease in blood pH and increase in lactate and peroxide caused by anaerobic exercise enhance EOF in horses (Hanzawa & Watanabe 2000). Erythrocyte malondialdehyde (MDA) concentration has been shown to be a biomarker of lipid peroxidation (Belge, Cinar & Selcuk 2003). Lipid peroxidation is the oxidative deterioration of polyunsaturated lipids, which leads to the production of a degraded product, called MDA (Belge *et al.* 2003). Lipid peroxidation has been shown to cause profound alterations in the structural organisation and function of cell membranes, including decreased membrane fluidity, increased membrane permeability, inactivation of membrane-bound enzymes and loss of essential fatty acids (Van Ginkel & Sevanian 1994), resulting in membrane damage and cell death (Belge *et al.* 2003). Draper and Hadley (1990a) showed that production of MDA *in vivo* increases with exposure to environmental oxidants.

The use of donkeys as pack animals is cheap and affordable for the transportation of goods, including farm produce, by farmers in the rural areas to the markets. Packing and trekking donkeys over a long distance, which is a form of exercise, may alter the animals' physiological parameters, especially during the harmattan season, established to be the most thermally stressful season in the Northern Guinea Savannah zone of Nigeria (Ayo *et al.* 1998; Ayo, Obidi & Rekwot 2011; Igono, Molokwu & Aliu 1982). Exposure of animals to stress has been demonstrated to induce an increase in free radicals in the body. Vitamin C, also known as ascorbic acid (AA), is a potent antioxidant, shown to scavenge free radicals in the body (Belge *et al.* 2003; Urban-Chmiel *et al.* 2009). The possible oxidative effect of packing in donkeys is unknown, especially during the thermally stressful harmattan season.

There are three seasons in the northern Guinea savannah zone of Nigeria, the cold-dry (harmattan) (November–February), hot-dry (March–April) and rainy (May–October) seasons (Ayo *et al.* 2011; Igono *et al.* 1982). The harmattan season is characterised by a cold dust-laden wind, with low ambient temperature in the morning and evening hours of the day, but high ambient temperature during the day (Ayo *et al.* 1998; Moberg, Esu & Malgwi 1991).



The aim of the study was to investigate the effect of packing on EOF of donkeys, and the role of AA.

Materials and methods

Experimental animals and management

Twelve healthy pack donkeys comprising 6 males and 6 non-pregnant females, aged 3–4 years and weighing between 98 kg and 100 kg served as subjects. The donkeys were obtained from the donkey market at Sheme, about 10 km from Faskari (11°43'N, 7°2'E), Nigeria. The donkeys were reared under traditional extensive management system without the provision of shelter (Minka & Ayo 2007). They were in good body condition with a score of 7 (Pearson & Ouassat 2000). The donkeys were fed with guinea corn straw and groundnut hay in the proportion of 4:1 and supplemented with wheat bran. In addition, 1 kg of whole sorghum grain was fed to each donkey per day throughout the experimental period. The donkeys were given access to water and salt-lick *ad libitum* during the experimental period.

The study area

The study was carried out during the harmattan season from 21st to 25th January, 2011. The study area covered an area from the Faculty of Veterinary Medicine, Ahmadu Bello University (ABU), Zaria (11°10'N, 7°38'E) to Panhauya village (11°7'N, 7°37'E), Kaduna State, located in the Northern Guinea Savannah zone of Nigeria. The donkeys covered a distance of 20 km (to and fro), from the Research Pen to Panhauya village on the outskirts of ABU, Zaria. The terrain of the route was narrow and stony, typical of the trekking route taken by pack donkeys in the zone.

Administration of ascorbic acid

The experimental animals ($n = 6$) were administered with AA (Sigma Chemicals, St. Louis, MO) orally and individually at 200 mg/kg (Chervyakov, Yevdokimov & Vishker 1977; Snow, Gash & Cornelius 1987), dissolved in 20 mL of sterile water. The control animals ($n = 6$) were given 20 mL of sterile water only. The administration was done by gavage 30 min before packing the donkeys on each day of the experiment.

Packing procedure

All the donkeys were saddled at 07h00 with a locally-made leather saddle pack frame, padded with chopped dry grasses to provide a cushion effect on the back of the animals. The saddles were loose enough to flap on both sides of the body of each donkey. Each donkey was subjected to work by packing it with sand, at the loading rate of 50% of live weight (Pal, Kumar & Gupta 2002) at 10h00 on each experimental day. The loads were balanced evenly with similar weight bulk on either side of the animal, and padding was arranged such that it was thickest along sides of the backbone (Oudman 2004). The donkeys were used for work according to the standard procedure for packing as described by Pal *et al.* (2002). They were subjected to work for three days; for 4 h (from 10h00 to 14h00) each day, and one day apart.

Blood sample collection

Blood was collected from the donkeys, pre-packing and post-packing at 06h00 and 14h00, respectively. Blood (4 mL) was aseptically collected from each donkey by jugular venipuncture, using a 10-mL syringe and 18-gauge sterile needles. The blood sample was divided into two (2 mL each): one part with anticoagulant for EOF test, and the other without anticoagulant to extract serum samples for the determination of MDA concentration.

Determination of erythrocyte osmotic fragility

Erythrocyte osmotic fragility was determined in saline (Sodium Chloride [NaCl]) solutions as described by Oyewale *et al.* (2011). Briefly, 5 mL of varying concentration of each test solution of NaCl (0.1%, 0.3%, 0.5%, 0.7%, 0.9%) was prepared in a set each of 5 centrifuge tubes. Blood (0.02 μ L) was added to each concentration of the test solution in each tube. The contents were mixed and incubated at room temperature for 30 min and then centrifuged at 3000 g for 10 min. The haemoglobin content of the supernatant was determined spectrophotometrically at a wavelength of 540 nm using the Bausch and Lomb spectrophotometer (FL Sales Inc. Grafton, Ohio, USA), with distilled water serving as blank. The percentage haemolysis in each concentration of NaCl was evaluated taking the tube with maximum haemolysis (0%) as 100%.

Determination of serum malondialdehyde concentration

The serum MDA was analysed using the method of Draper and Hadley (1990b). Briefly, 2.5 mL of 100 g/L trichloroacetic acid solution was added to 0.5 mL serum in each centrifuge tube and placed in a boiling water bath for 15 min. After cooling in tap water, the mixture was then centrifuged at 1000 g for 10 min, and 2 mL of the supernatant was added to 1 mL of 6.7 g/L thiobarbituric acid 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 ultraviolet (UV) spectrophotometer (T80+ UV/VIS Spectrometer, PG instruments Ltd., Alma Park, Wibtoft, Leicestershire, LE 175BE, UK) at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex (1.56×10^5 /cm), and expressed in μ mol/mL.

Statistical analysis

The data obtained were expressed as mean \pm standard error of the mean (Mean \pm SEM). Malondialdehyde data were subjected to one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison *post-hoc* test, using GraphPad Prism 4.0 for Windows (GraphPad Software, San Diego, California, USA). The EOF data were subjected to Student's *t*-test to compare the differences between the means, obtained from the control and experimental donkeys. Values of $P < 0.05$ were considered significant.

Ethical considerations

The handling, packing and trekking of the donkeys were carried out humanely in accordance with the guidelines, governing the welfare of research animals by the Ahmadu

Bello University, and as approved by the Ethics Research Committee of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria under the permit number 5154.

Results

Erythrocyte osmotic fragility

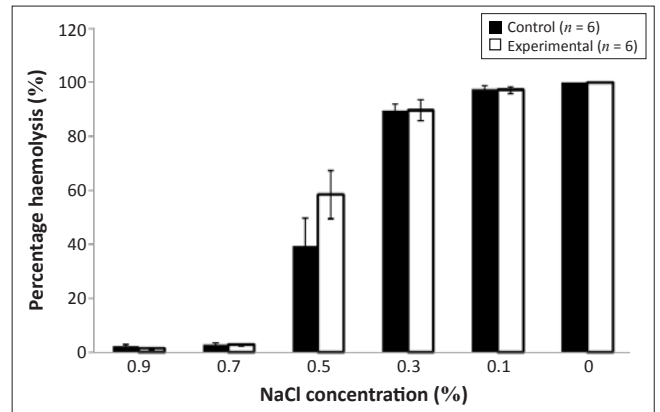
The results of EOF are shown in Figures 1 and 2. Pre-packing, the percentage haemolysis in the experimental group was not significantly ($P > 0.05$) different from values obtained in the control group at all the different NaCl concentrations (Figure 1). Post-packing, the percentage haemolysis in the experimental group was not significantly ($P > 0.05$) different from that of the control group at different NaCl concentrations, except at 0.3% NaCl concentration. At 0.3% concentration, the percentage haemolysis of $93.69\% \pm 2.21\%$ obtained in the control donkeys was significantly ($P < 0.05$) higher than the value of $71.31\% \pm 8.33\%$, recorded in the experimental donkeys (Figure 2).

Malondialdehyde concentration

The changes in serum MDA concentration in the pack donkeys were also calculated (Figure 3). In the control donkeys, the pre-packing serum MDA concentration significantly ($P < 0.05$) rose from pre-packing value of $30.03 \mu\text{mol} \pm 3.42 \mu\text{mol}$ to post-packing value of $39.62 \mu\text{mol} \pm 4.16 \mu\text{mol}$. Similarly, in experimental donkeys MDA increased significantly ($P < 0.05$) from pre-packing value of $26.34 \mu\text{mol} \pm 2.17 \mu\text{mol}$ to post-packing value of $35.97 \mu\text{mol} \pm 2.88 \mu\text{mol}$. However, the post-packing MDA concentration of $39.62 \mu\text{mol} \pm 4.16 \mu\text{mol}$, obtained in the control donkeys was not significantly ($P > 0.05$) different from the value of $35.07 \mu\text{mol} \pm 2.88 \mu\text{mol}$ recorded in the experimental donkeys.

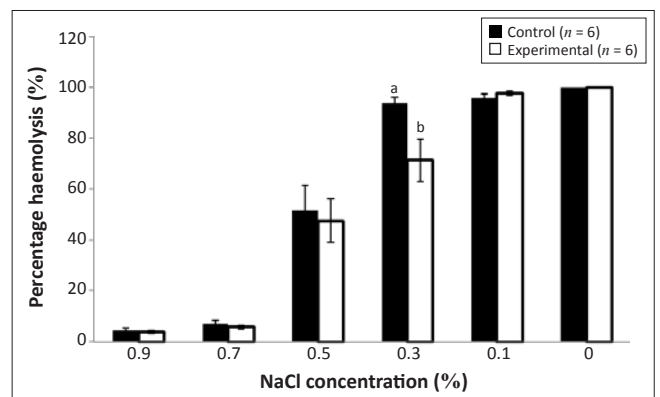
Discussion

The result of erythrocyte osmotic fragility showed that packing increased haemolysis in donkeys and the effect was more evident in the control than experimental donkeys. This agrees with the findings of Hesta *et al.* (2008), who showed that physical exertion increases haemolysis. The increased haemolysis obtained in the present study post-packing may be attributed to the effects of stress due to packing. This finding is in agreement with that obtained by Nazifi *et al.* 2009, who demonstrated that stress due to transportation induced oxidative stress resulting in an increase in the generation of free radicals. It has been established that erythrocytes are very susceptible to stress (Agrawal & Sharma 2010; Altan *et al.* 2003; Vani, Shiva Shankar & Asha Devi 2010). Therefore, the mitigation of packing-induced haemolysis by AA may be due to its antioxidant effect. Tauler *et al.* (2003) demonstrated that AA is capable of stabilising the integrity of cell membranes and decreasing its susceptibility to lipid peroxidation in exhaustive exercise, a form of stress. The result of the present study demonstrated for the first time that EOF may serve as a biomarker of stress due to packing in donkeys, and may be an important diagnostic tool for evaluating post-packing stress in donkeys.



NaCl, Sodium Chloride.

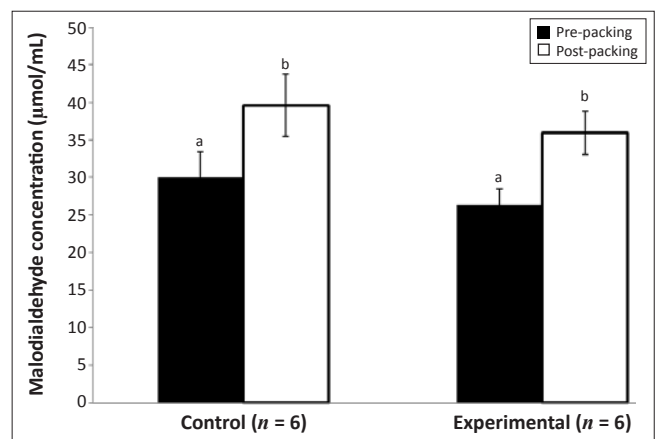
FIGURE 1: The erythrocyte osmotic fragility of the experimental and control donkeys pre-packing during the harmattan season.



NaCl, Sodium Chloride.

^{a,b}, Values with error bars having different superscript letters are significantly ($P < 0.05$) different.

FIGURE 2: Effect of ascorbic acid on the erythrocyte osmotic fragility of donkeys subjected to packing during the harmattan season.



^{a,b}, Values with error bars having different superscript letters are significantly ($P < 0.05$) different.

FIGURE 3: Changes in malonaldehyde concentration in donkeys subjected to packing during the harmattan season.

The increased MDA concentration obtained in the control donkeys and experimental donkeys after packing occurred, apparently, as a result of free-radical induced lipid peroxidation of the erythrocyte membranes, which impairs cell integrity and results in cell destruction (Hanzawa & Wantanabe 2000; Jain 1986). There was no significant decrease in post-packing MDA concentration in the experimental



donkeys when compared with that of control donkeys, indicating that AA did not ameliorate lipid peroxidation in donkeys, subjected to the packing. The result disagreed with previous findings that AA supplementation ameliorates oxidative damage due to heat stress in donkeys (Dey *et al.* 2010; Lin, Decuypere & Buyse 2006), chemical-induced stress in mice (Ambali *et al.* 2010a, 2010b) and humans (Tauler *et al.* 2003), but agreed with the findings of Hesta *et al.* (2008) who reported that AA had no effect on thiobarbituric acid in dogs. However, in contrast to the finding of the present study, the dogs were not subjected to exercise. The result of MDA concentration obtained in the present study shows that EOF may be of better value in the determination of post-packing stress than MDA concentration, and may be a better biomarker of oxidative stress in donkeys subjected to packing. The finding of the present study also disagreed with the result of Aydemir *et al.* (2000) that AA strongly reduces MDA concentration in erythrocytes of chickens. The differences in the results may be due to species and especially the high resistance of donkeys to unfavourable environmental conditions (Fielding & Krause 1998). Although AA has been shown to exert its antioxidative effect by removing singlet oxygen, hydroperoxyl, superoxide, lipid peroxyl and lipid free radicals (Belge *et al.* 2003), and thus decreases MDA concentration in animals subjected to stress, the packing regimen used in the present study was, apparently, not stressful enough to induce increased oxidative stress in the donkeys. The difference in the results obtained in the present study and those of other investigators may be due to the hardy nature and high adaptation of the donkey, the weather during which the study was carried out, and the short duration of work done by the pack donkeys. This requires further investigation.

Conclusion

Packing induced work stress in donkeys, resulting in an increase in haemolysis, which was ameliorated by AA administration. It is recommended that AA should be administered to donkeys before packing in order to ameliorate the adverse effects of work stress.

Acknowledgements

We wish to thank the team of reviewers for their tireless effort in improving the manuscript.

Competing interests

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this paper.

Authors' contributions

J.O.A. (Ahmadu Bello University) was the project leader responsible for experimental and project design and wrote the manuscript. S.F.A. (Ahmadu Bello University) and P.I.

(Ahmadu Bello University) made conceptual contributions. F.O. (Ahmadu Bello University) performed most of the experiments, prepared the samples, did the calculations and wrote the manuscript.

References

- Adenkola, A.Y. & Ayo, J.O., 2009, 'Effects of road transportation on erythrocyte osmotic fragility administered ascorbic acid during the harmattan season in Zaria, Nigeria', *Journal of Cell and Animal Biology* 3(1), 4–8. PMID:15590494
- Adenkola, A.Y., Ayo, J.O., Sackey, A.K.B. & Adelaiye, A.B., 2010, 'Erythrocyte osmotic fragility of pigs administered ascorbic acid and transported by road for short-term duration during the harmattan season', *African Journal of Biotechnology* 9(2), 226–233.
- Agrawal, A. & Sharma, B., 2010, 'Pesticides induced oxidative stress in mammalian systems: A review', *International Journal of Biological and Medical Research* 1(3), 90–104.
- Altan, O., Pabuccuoglu, A., Koyanlioglu, S. & Bayraktar, H., 2003, 'Effect of heat stress on oxidative stress, lipid peroxidative and some stress parameters in broilers', *British Poultry Science* 44(4), 545–550.
- Ambali, S.F., Akanbi, D., Shittu, M., Giwa, A., Oladipo, O.O. & Ayo, J.O., 2010a, 'Chlorpyrifos-induced clinical, haematological and biochemical changes in Swiss albino mice: Mitigating effect by co-administration of vitamins C and E', *Life Science Journal* 7(3), 37–44.
- Ambali, S.F., Ayo, J.O., Ojo, S.A. & Esievo, K.A.N., 2010b, 'Ameliorative effect of vitamin C on chronic chlorpyrifos-induced erythrocyte osmotic fragility in Wistar rats', *Human and Experimental Toxicology* 30(1), 19–24.
- Asala, O.O., Ayo, J.O., Rekwot, P.I., Minka, N.S., Omoniwa, D.O. & Adenkola, A.Y., 2011, 'Effect of ascorbic acid administration on erythrocyte osmotic fragility of pigs transported by road during the hot-dry season', *Veterinary Research Communications* 35(3), 245–254.
- Aydemir, T., Ozturk, R., Bozkaya, L.A. & Tarhan, L., 2000, 'Effects of antioxidant vitamins A, C, E and trace elements Cu, Se on CuZn SOD, GSH-Px, CAT and LPO levels in chicken erythrocytes', *Cell Biochemistry and Function* 18(2), 109–115.
- Ayo, J.O., Obidi, J.A. & Rekwot, P.I., 2011, 'Effects of heat stress on the well-being, fertility and hatchability of chickens in the northern Guinea savannah zone of Nigeria: A review', *ISRN Veterinary Science* Art. #838606, 10 pages.
- Ayo, J.O., Oladele, S.B., Fayomi, A., Jumbo, S.D. & Hambolu, J.O., 1998, 'Body temperature, respiratory and heart rate in the red Sokoto goat during the harmattan season', *Bulletin of Animal Health and Production in Africa* 46(2), 161–166.
- Belge, F., Cinar, A. & Selcuk, M., 2003, 'Effects of stress produced by adrenocorticotropin on lipid peroxidation and some antioxidants in vitamin C treated and non-treated chickens', *South African Journal of Animal Science* 33(3), 201–205.
- Chervyakov, D.K., Yevdokimov, P.D. & Vishker, A.S., 1977, *Drugs in veterinary medicine*, Kolos Publishing House, Moscow (in Russian).
- Dey, S., Dwivedi, S.K., Malik, P., Panisup, A.S., Tandon, S.N. & Singh, B.K., 2010, 'Mortality associated with heat stress in donkeys in India', *The Veterinary Record* 166(5), 143–145.
- Draper, H.H. & Hadley, M., 1990a, 'A review of recent studies on the metabolism of exogenous and endogenous malondialdehyde', *International Society for the Study of Xenobiotics* 9(6), 901–907.
- Draper, H.H. & Hadley, M., 1990b, 'Malondialdehyde determination as index of lipid peroxidation', *Methods in Enzymology* 186, 421–431.
- Fielding, D. & Krause, P., 1998, *The tropical agriculturist – Donkeys*, Macmillan, London and Basingstoke.
- Hanzawa, K. & Wantanabe, S., 2000, 'Changes in osmotic fragility of erythrocytes during exercise in athletic horses', *Journal of Equine Science* 11(1), 51–61.
- Hesta, M., Ottermans, C., Krammer-Lucas, S., Zentek, J., Helweg, P. & Buyse, J., 2008, 'The effect of vitamin C supplementation in healthy dogs on antioxidative capacity and immune parameters', *Journal of Animal Physiology and Animal Nutrition* 93(1), 26–34.
- Igono, M.O., Molokwu, E.C.I. & Aliu, Y.O., 1982, 'Body temperature responses in savannah brown goats to the harmattan and hot-dry seasons', *International Journal of Biometeorology* 2(1), 225–230.
- Jain, N.C., 1986, *Schalm's veterinary haematology*, 4th edn., Lea and Febiger, Philadelphia, viewed 23 June 2011, from <http://books.google.com/books?isbn=0683306928>
- Lin, H., Decuypere, E. & Buyse, J., 2006, 'Acute heat stress induces oxidative stress in broilers', *Comparative Biochemistry and Physiology* 144(1), 11–17.
- Minka, N.S. & Ayo, J.O., 2007, 'Physiological responses of goats treated with ascorbic acid during the hot-dry season', *Animal Science Journal* 78(2), 164–172.
- Minka, N.S. & Ayo, J.O., 2010, 'Physiological responses of erythrocytes in goats to transportation and the modulatory role of ascorbic acid', *Journal of Veterinary Medical Science* 72(7), 875–881.
- Moberg, J.P., Esu, I.E. & Malgwi, W.B., 1991, 'Characteristics and constituent composition of harmattan dust falling in northern Nigeria', *Geoderma* 48(2), 73–81.
- Nazifi, S., Saeb, M., Baghshani, H. & Saeb, S., 2009, 'Influence of road transportation during hot-summer condition on oxidative status biomarkers in Iranian dromedarian camels (*Camelus dromedarius*)', *African Journal of Biochemistry Research* 3(7), 282–287.



- Oudman, L., 2004, *Donkeys for traction and tillage*, Digigrafi, Wageningen.
- Oyewale, J.O., Dzenda, T., Yaqub, L., Akanbi, D., Ayo, J., Owoyele, O. *et al.*, 2011, 'Alterations in the osmotic fragility of camel and donkey erythrocytes caused by temperature, pH and storage', *Veterinarski Arhiv* 81(4), 459–470.
- Pal, Y., Kumar, S. & Gupta, A.K., 2002, 'Blood gases, acid-base and physiological indices in donkeys as pack animals', *Draught Animal News* 37(2), 27–33.
- Pearson, R.A. & Ouassat, M., 2000, *A guide to liveweight estimation and body condition scoring of donkeys*, Centre for Tropical Veterinary Medicine, University of Edinburgh, Edinburgh.
- Snow, D.H., Gash, S.P. & Cornelius, J., 1987, 'Oral administration of ascorbic acid to horses', *Equine Veterinary Journal* 19(2), 520–523.
- Tauler, P., Aguilo, A., Gimeno, I., Fuentespina, E., Tur, J.A. & Pons, A., 2003, 'Influence of vitamin C diet supplementation on endogenous antioxidant defense during exhaustive exercise', *European Journal of Physiology* 446(6), 658–664.
- Urban-Chmiel, R., Kankofer, M., Wernicki, A. & Puchalski, A., 2009, 'The influence of different doses of α -tocopherol and ascorbic acid on selected oxidative stress parameters in *in vitro* culture of leucocytes isolated from transported calves', *Livestock Science* 127(3), 365–370.
- Van Ginkel, G. & Sevanian, A., 1994, 'Lipid peroxidation induced membrane structural alterations', *Methods in Enzymology* 233, 273–288.
- Vani, R., Shiva Shankar, R.C.S. & Asha Devi, S., 2010, 'Oxidative stress in erythrocytes: A study of an effect of antioxidant mixtures during intermittent exposures to high altitude', *International Journal of Biometeorology* 54(5), 553–562.