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Review

Physiological responses of donkeys (*Equus asinus*, Perissodactyla) to work stress and potential ameliorative role of ascorbic acid

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The review examines donkey as a draught animal, used mainly for carrying load from one place to another in Africa. Although, motorized transport has been thriving rapidly in the twentieth century, the use of donkeys for transport has not experienced a proportionate decline. Donkeys are often overused, neglected and exposed to various stressors, which eventually lead to their poor performance. This review also examines the effect of packing on the physiological parameters of donkeys and the ameliorative role of ascorbic acid in donkeys subjected to work stress. In conclusion, a better understanding of the pathophysiologic mechanism of stress in donkeys may enhance their work output under the unfavourable environmental conditions of the tropics.

Key words: Donkeys, ascorbic acid, packing, stress, physiological parameters.

INTRODUCTION

The modern domestic donkey (*Equus asinus, Perissodactyla*) was bred from the wild ass (*Equus africanus*) in north-eastern Africa during the predynastic period of Egypt, about 6 000 years ago. Two wild ass sub-species are thought to have had a role in the development of the modern donkey. They are the Nubian (*Equus africanus africanus*) and Somali (*Equus africanus somaliensis*) donkeys. Recent DNA analysis suggested that only the Nubian ass contributed genetically to the domestic donkey (Kimura et al., 2010). The early history of the donkey in Africa is difficult to reconstruct because of the sparse archaeological data. Draught animals, the principal being donkeys, play an important role in agricultural production and transport in sub-Saharan Africa. Although draught animal power has been superseded by tractors on many large commercial farms in Africa, it is a relevant farm technology in small-scale agriculture for economic and agro-ecological reasons (Pearson and Vall, 1998). Nigeria, with a donkey population of one million is ranked third in Africa after Ethiopia and Egypt (Blench, 2003).

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Donkeys are preferred as draught and transport animals because they are friendly, hardy, quiet and more economical, compared to horses and oxen. They can be maintained on local farm produce, and are easy to train, intelligent and patient while working (Inns, 1979). Donkeys as pack animals can carry a load that is up to 100 kg or that is 50% of their body weight (Pal et al., 2002). It has also been reported that donkeys can carry a heavy load twice the weight a person can carry for a longer distance (Martin and Smith, 2005). Donkeys are major assets to human lives in arid areas, but their physical needs are always ignored due to their hardiness. It is important to protect donkeys from harsh elements of the weather in order to improve their health (Kataria and Kataria, 2010). As donkeys become a more popular choice of work animals for small-holder farmers, specific management practices need to be devised in order to fully maximize their natural survival advantages (Smith and Pearson, 2005). The improvement in health and welfare will enhance their productivity and provide their owners with a more reliable source of income (Martin et al., 2005). A better understanding of the pathophysiologic mechanism of stress in donkeys may enhance their work output under the unfavo-

unfavorable environmental conditions of the tropics. From a welfare point of view, the use of ascorbic acid may provide a less expensive and readily available alternative to other anti-stress agents. This is particularly important, if they can be shown to remove or at least mitigate the harmful consequences of stress responses in the donkey.

ADAPTATION OF THE DONKEY

Origin of the wild ass in Africa is controversial, but it may have originated outside the sub-saharan regions of Africa. However, the true home of the donkey is the arid and semi-arid areas of North-East and Western Asia (Fielding and Krause, 1998). According to Fielding and Krause (1998), donkeys adapt well to hot-dry desert environment through body temperature control, water metabolism, and special nutritional and anatomic features. Donkeys in the tropics are able to maintain homeothermy by compensatory mechanisms in order to keep their physiological values within the established normal range (Minka and Ayo, 2007). This despite the prevailing high ambient temperature (AT) (Minka and Ayo, 2007a), is established to induce heat stress in other livestock (Ayo et al., 1996, 2008).

The most limiting factor for survival in semi-arid and arid areas and during drought is water. Donkeys survive better than cattle in these areas and during drought (Homer 1993; Smith and Pearson, 2005). They have the ability to tolerate thirst and this allows them to have access to more remote source of forage, inaccessible to cattle in rangeland (Smith and Pearson, 2005). Nengomasha et al. (2000) reported that donkeys with limited access to water (2 to 3 days) loose less water through faeces than their counterparts with *ad libitum* access to water. This is because faecal water loss can account for 50% of all water lost from the body. Donkeys are also adapted to low-quality, high-fibre feed, which contributes to their ability to eat and survive on very little quantity (Yousef, 1991; Nengomasha et al., 2000). Donkeys do very well in selecting their feed and it is better to feed them *ad libitum* when given poor-quality cereal straw diets and stovers to improve the feeding value (Pearson and Meritt, 1991). Pearson et al. (1998), however, reported that donkeys have the tendency to overeat when they have access to good quality roughages. Fielding and Krause (1998) reported that the positioning of the stomach avoids food from being washed out of it by water when donkeys drink. They have tough, compact hooves that have successfully adapted to walking on sandy and rocky terrain. Donkeys have wooly hair which insulates them from desert heat and cold and a lean body mass that is fuel-efficient and easily cooled. Socio-economically, owing to their single-purpose role and their low social status, maintenance of a low sustainable population of donkeys is possible (Smith and Pearson, 2005).

SPECIAL PHYSIOLOGICAL FEATURES OF THE DONKEY COMPARED TO THE HORSE

The physiology of the donkey differs in some aspects from horses and mules. Normal base-line values for most hematology and biochemical indices have been published for donkeys and are slightly different from those of horses (Grosenburgh et al., 2011). For instance, there is absence of reticulocytes in peripheral circulation, even after severe hemorrhage in donkeys. Donkeys usually have fewer, but larger erythrocytes.

Hypothermia in donkeys is defined as having rectal temperature below 35°C during cold period and may not be secondary to other diseases related to diet or management. Donkeys are thermolabile, and so their temperature may increase more in a hot climate, or following exercise than for a normal horse. Normal values of resting heart rates are higher in donkeys than for a normal horse, and heart rate is a very good indicator of stress or pain, even when it is not noticed physically in donkeys. The donkey's fluid balance and water compartment partitioning differ from those of the horse, thus they tolerate dehydration better than the horse and recover quickly (Grosenburgh et al., 2011). The hematocrit increase secondary to dehydration does not occur in donkeys until they are 30% dehydrated.

USES OF DONKEYS FOR TRACTION

Animal traction can be defined as the employment of domestic animals for tillage or transport (Starkey, 1992). After cattle, the main work animals world-wide are equids. The real importance of the donkeys relates to their use as pack animals because they are adapted to the desert and can carry loads through arid lands. Donkeys have been employed for packing over 5 000 years ago (Clutton-Brock, 1992). Donkeys as pack animals, are used to carry people, water, manure, firewood, farm inputs and produce, and to relieve women of much of their time and energy-consuming work (Pearson, 2000; Minka and Ayo, 2007a; Ayo et al., 2008).

In Africa, while motorized transport has been thriving rapidly since the twentieth century, the use of animal power for local transport has not experienced a proportionate decline. Donkeys have been important in the history of salt trade in Ethiopia (Wilson, 1991). In West-Africa, donkeys were not widespread until about 1 500 years ago, and ever since then, they have been used mostly as pack animals (Blench, 1993). The use of donkeys for transportation continues, especially in rural areas with rugged terrain, absence of well-developed modern transport network and prevailing poverty (Mengistu et al., 2005) and the contribution of donkeys in facilitating agricultural production is very high (Youseph et al., 2005). Animal traction is socially accepted with no taboos and there is a supplementary and complementary connection between the crop sector and draught animals (Djang-Ford jour et al., 2003). It has been predicted that animal traction will still spread in most sub-Saharan Africa even in the foreseeable future.

STRESS IN DONKEYS

Donkeys in Nigeria are used for transport purposes in different parts of the country, especially in places with difficult terrain. Under this condition, donkeys are subjected to different stressors, which may compromise their health and consequently, result in poor performance (Gupta et al., 1999). Stress generally affects the host-defense system and increases its susceptibility to infections (von Borell, 2001). The different conditions by which donkeys are subjected to stress are enumerated below.

Work stress

Work is produced when animals by physical exertion use energy to pull an implement through the soil or to carry a load over a distance (Pearson and Vall, 1998). The amount of work an animal can do depends on the speed at which it works, and the draught force generated. For a particular draught force, it is the speed that determines the power output of the animal. Therefore, as draught force increases, speed correspondingly decreases (Francis, 1988). Adverse working conditions, including high ambient temperature and relative humidity resulting in heat stress, badly fitted harness and difficult ground conditions, are stressors that reduce the rate of work (Pearson and Vall, 1998). Thus, work output is not just a function of the speed of the animal itself, but is influenced by the environment and soil conditions (Pearson and Vall, 1998). The oxidation of chemical energy to provide work produces heat, which has to be lost by the animal, if it is to continue to work and maintain homeostasis. However, donkeys can still work while withstanding severe dehydration (Youseph, 1991). They do this by reducing water intake, sweating rate and water excretion, and by maintaining feed-intake. They also maintain plasma volume by drawing substantial fluid reservoir in the hind-gut, especially the caecum and ventral colon. Mild chronic dehydration experienced by the donkey produces morphological and physiological adaptations in the hind-gut, which enhance fermentation and absorptive capacity of the hind-gut (Sneddon et al., 2006). With their good adaptability, in hot climate, heat loss in donkeys has been observed. Olaifa et al. (2012a) observed an increase in rectal temperature in donkeys after a 4 h load carrying. Rose (1986) reported that horses exercising over a distance of 100 km may lose 30 to 40 L of water in sweating during the course of the exercise. Pal et al. (2002) observed signs of fatigue, which include unwillingness to continue, uncoordinated legs and excitement after work, and concluded that continuous working of donkeys causes increased stress.

Thermal environmental stress

Meteorological stressors that directly and adversely affect livestock performance and health care, especially in tropics and sub-tropical Africa are AT, relative humidity (RH), thermal radiation and wind speed (Ayo et al., 2011). Smith et al. *(*1995) shows that the meteorological stressors exert adverse effects on animal health, growth, production and overall performance. AT is positively correlated with the rectal temperature of donkeys during the harmattan season (Olaifa et al., 2012a) and this is because exposure to high ambient temperature induces the animal to attempt to balance the excessive heat load by using thermoregulatory mechanisms to dissipate their latent heat. The temperature begins to rise if the mechanism fails. Of all the meteorological stress factors adversely affecting livestock, heat stress factors have been shown to be the most severe in the tropics (Ayo et al, 1996). Heat stress is always a potential problem, which causes obvious discomfort, tremors and rapid shallow breathing in donkeys (Fielding and Krause, 1998). Heat stress causes cascade of changes in the physiological mechanisms of animals. In donkeys, Kataria and Kataria (2010) reported an increase in prolactin and cortisol levels, suggestive of stress adaptation to unfavorable environment.

Although there is paucity of information on the effect of cold stress on the donkey, especially in the tropics, stress due to cold environment or low ambient temperature may be a potential problem too, especially during the harmattan season, in the early hours of the day (Ayo et al., 1998). It may be reflected in an elevated metabolic rate and increased production of reactive oxygen species (Heise et al., 2003). Cold stress has been shown to disrupt the balance in the oxidant/antioxidant system, and it causes oxidative damage to several tissues by altering the enzymatic and non-enzymatic antioxidant status, protein oxidation and lipid peroxidation (Sahin and Gusmulu, 2004).

Poor management

Improper handling is considered as a major stressor, adversely affecting farm animals (Knowles and Warris, 2000; Minka and Ayo, 2009). It exerts deleterious effects on health, well-being, behavior, performance and production quality (Geverink et al., 1998). Improper housing and handling expose donkeys to wounds, which are commonly not attended to and leading to poor performance. Donkeys are exposed to wound infections mostly during the hot-dry and rainy seasons (Asha et al., 2006). They are commonly fed with poor-quality feed, where proper feeding of donkeys enables them to resist better to disease, live longer and have a higher rate of reproducetion to provide replacement animals. Besides, donkeys need supplements, especially throughout the dry season, when they are increasingly used for traction (Olaifa et al., 2012a).

BIOMARKERS OF STRESS IN DONKEYS

Rectal temperature, respiratory rate and heart rate

It has been established that rectal temperature, respiratory rate and heart rate are relevant body parameters for immediate evaluation of health status and adaptability of animals (Ayo et al., 1996, 2008; Minka and Ayo 2007a; Dey et al., 2010). They can be measured easily, especially during transportation in rural areas (Minka and Ayo, 2007c). Continuous packing for 4 h has been reported to induce a transient increase in rectal temperature (Olaifa et al., 2012a) and respiratory rate in donkeys (Pal et al., 2002). High body temperature initiates compensatory and adaptive mechanisms to establish the body's homeostasis and homeothermy (Stott, 1981). Donkeys adapt well to different environmental conditions by compensatory increase in values of rectal temperature, respiratory rate and heart rate (Minka and Ayo, 2007a). Increase in these physiological parameters above the normal range indicates that the animals are stressed. This has been confirmed in sheep (Srikandakumar et al., 2003), donkeys (Pal et al., 2002; Minka and Ayo, 2007a) and goats (Ayo et al., 1996). In donkeys, rectal temperature, respiratory rate and heart rate are stress quantifiable parameters, used to define stress levels under continuous work (Singh, 2000). The standard ranges used to define levels of stress according to Singh (2000) are as follows:

Rectal temperature (ºC) = $T_0 + (1.0 – 3.0)$
Heart rate (beats per minute) = $H_0 + (15 – 45)$
Respiratory rate (breaths per minute) = $R_0 + (15 - 50)$;

Where T_0, H_0 and R_0 parameters are recorded at rest. In an experimental study carried out by Olaifa et al. (2012a), a significant increase in rectal temperature was observed in donkeys following packing. The result support the hypothesis that donkeys subjected to packing are often stressed.

BEHAVIORAL RESPONSES

Behavioral responses are the first line of defense to environmental challenges (Ayo et al., 2002; Cable et al., 2007). When the brain perceives a stressful experience, it initiates physiologic and behavioral responses that lead to allostasis and adaptation (Armario et al., 2008). The hypothalamic-pituitary-adrenal axis and the sympathetic adrenomedullary system have a crucial function in the metabolic and cardiovascular preparation of the body to exhibit certain behavioral responses (Sapolsky et al., 2000). Stress responses in donkeys are always conducted on the basis of irregular behavioral phenomena that may be difficult to interprete as observed in previous works (Minka and Ayo, 2007a; Ayo et al., 2008). The perception of stress is influenced by experience, genetics and behavior. A major problem of applied stress studies in animals relates to the methodology of stress assessment or to the non-specificity of the selected variable (von Borell, 2001). Laedwig (2000) reveals that animals, like donkeys, under intensive housing conditions, exhibit chronic intermittent stress response, occurring in situations such as standing up or lying down in inadequately designed cubicle or tether stalls. The response of the donkey to a stressor also depends on temperament and early experience. It has been debated that farm animals develop distinct coping strategies as demonstrated in laboratory animals. Grandin (1997) demonstrated that excitability of animals depend on their temperament. The more the animals are handled, the aggressive they become and conversely, frequent handling of calm animals result in increase in their aggression (von Borell, 2001). In a study by Minka and Ayo (2007a), unshaded donkeys subjected to work during the hot-dry season exhibited behavioral values; lower frequency of lying down and lower speed compared to shaded donkeys.

HEMATOLOGY AS A STRESS BIOMARKER

Carrying load over a long distance is a strenuous exercise for donkeys (Pearson and Vall, 1998) thus; this can influence their hematologic parameters due to work stress (Lorena et al., 2006). The sympathetic nervous system generally plays an important role in the mediation of response to exercise by modifying cardiovascular function (Cuniberti et al., 2010), and thereby affecting the responses that are observed in blood parameters. Changes occurring in hematologic parameters induced by physical work have been well documented (Hindchiff et al., 2002; Pal et al., 2002; Lorena et al., 2006).

Erythrocytes

Erythrocytes are naturally prone to oxidative stress because of the high content of polyunsaturated fatty acid in their membranes and high amount of hemoglobin-bound iron (Agrawal and Sharma, 2010). Erythrocytes are an ideal model for studying the effects of physical stress since they lack nucleus and, have a short lifespan (Vani et al., 2010). Many *in vitro* and *in vivo* studies have demonstrated that several parameters of erythrocytes are negatively affected by increased oxidative stress (Brzezinska-Slebodzinska, 2003; Bhatti and Shaikh, 2007; Pandey and Risvi, 2009), produced following physical exercise, which is a form of stress. Bhatti and Shaikh (2007) reported an increase in erythrocyte count following physical exercise and slight increase in erythrocyte sedimentation rate. They may increase apparently, due to inflammatory components and release of acute phase protein in the liver cells. Increase in total number of circulating erythrocytes, hematocrit and hemoglobin concentration has been observed (Boning et al., 2010; Cuniberti et al., 2010) and may be due to splenic contractions during exercise. The spleen acts as a reservoir for erythrocytes, storing a large volume of blood, about 40 to 50% of the entire circulatory population, and the release of erythrocytes is under the influence of sympathetic nervous system and circulating catecholamines (Cuniberti et al., 2010).

Erythrocyte osmotic fragility

Erythrocyte osmotic fragility is also a good indicator of work stress (Hesta et al., 2008). Increase in erythrocyte osmotic fragility has been reported in donkeys after subjecting them to packing (Olaifa et al., 2012b). Changes in the erythrocyte osmotic fragility during exercise in equine are an important indicator of intravascular hemolysis (Hanzawa and Watanabe, 2000). Erythrocyte osmotic fragility is used to determine the integrity of erythrocytes (Asala et al., 2011). It is reported that heavy exercise increases erythrocyte osmotic fragility while light exercise decreases it because light exercise has antioxidant properties (Cuniberti et al., 2010). The decrease in blood pH and increase in lactate and peroxide caused by anaerobic exercise promote erythrocyte osmotic fragility. Oyewale et al. (2011) reported that donkey erythrocyte is less resistant to osmotic hemolysis than that of other equids.

Leukocytes

Responses of leukocytes and their sub-populations to physical work are well established (McCarthy and Dale, 1988; Gabriel et al., 1992; Pederson and Hoffman-Goetz, 2000; Bhatti and Shaikh, 2010). Pederson and Hoffman-Goetz (2000) demonstrated that neutrophils rose during and after physical exercise, but a fall below basal values results after a prolonged exercise. Lorena et al. (2006) also reported an increase in leukocyte counts after marathon race in humans. On the contrary, Cuniberti et al. (2010) did not observe any change in total leukocyte counts after prolonged exercise, although they recorded neutrophilia, lymphopenia and monocytosis similar to the findings of Robson et al. (2003) in horses. The change in differential leukocyte count has been attributed to a rise in the concentration of hormones (that is adrenaline, cortisol and growth hormone) during exercise and increased cardiac output, which increases mobilization of leukocytes from marginated pool (Tonnesen et al., 1987). Exercise-induced leukocytosis has been often compared to inflammation-like reaction. After strenuous work, the leukocyte count is almost double, the baseline similar to the response against physiological insults to the immune system (Bhatti and Shaikh, 2007).

Neutrophil : lymphocyte ratio as an index of stress

Neutrophil : lymphocyte ratio is a good indicator of stress (Altan et al., 2003) as the ratio increases in stressed individuals (Plyaschenko and Sidorov, 1987). Minka et al. (2004) demonstrated an increase in neutrophil/lymphocyte ratio in pullets following road transportation stress due to inflammatory responses. Similarly, in donkeys, an increase in neutrophil/lymphocyte ratio was observed after work (Olaifa et al., 2012c).

BIOCHEMICAL RESPONSES

The parameters are usually evaluated to determine the extent of stress include electrolytes (Na^+, K^+, Cl^-, H^+) and serum enzymes such as aspartate aminotransferase, alkaline phosphatase, creatinine kinase, superoxide dismutase, catalase, glutathione peroxidase (GSH) and molecules of urea, glucose and total protein (Ambali et al., 2007; Lemma and Morges, 2009). Acids and bases are usually added continuously to the body fluids as a result of either ingestion or production during cellular metabolism (Srikandakumar et al., 2003). However, environmental and animal factors as well as thermoregulatory mechanisms exert significant effect on the energy exchange between the animal and the environment leading to changes, occurring in the normal acid-base balance. In order to combat these changes, the body utilizes three basic mechanisms, which are chemical buffering and respiratory adjustment of blood carbonic acid as well as excretion of H^+ or HCO_3^- by the kidneys (Srikandakumar et al., 2003). Heat stress has been known to cause respiratory alkalosis as a result of hyperventilation and compensation, which often result in urinary HCO_3^- loss in an attempt to balance the ratio of carbonic acid to bicarbonate in the blood (Benjamin, 1978).

Na^+ and K^+ have significant effects on the hydration status of the body system. Increased extracellular fluid osmolality (increased sodium concentration) or decreased K^+ plasma concentration indicates dehydration, which may eventually stimulate drinking mechanism (Hindchiff et al., 2002). During heat stress or dehydration, there is reduced supply of blood to the kidneys, because heat stress causes peripheral vasodilation in order to lose heat (Srikandakumar et al., 2003). All serum biochemicals, except total protein, have been reported to be significantly affected by workload in donkeys (Lemma and Moges, 2009). During exercise, water, Na^+, K^+ and Cl^- are lost in sweat and this may lead to electrolyte imbalance (Hinchdiff et al., 2002). Na^+, Cl^-, K^+ and bicarbonate are responsible for maintaining electrical neutrality and extracellular fluid by exerting enough osmotic pressure during low water intake. Donkeys subjected to stress due to feed deprivation did not show any significant change in these ions (Gupta et al., 2011). In the study conducted by Gupta et al. (2011), the response of donkeys was low ingestion of water and feed and was less severe in donkeys than the mules because of the lighter body weight and lesser nutrient requirement in the donkeys compared to the mules. Donkeys subjected to different workload and different categories of body condition have been known to show variations in concentrations of most serum biochemical compounds. The variations have been attributed to differences in metabolic and electrolyte regulatory mechanisms that control their concentrations in plasma and extracellular fluid (Lemma and Morges, 2009).

Malondialdehyde

Malondialdehyde concentration is a biomarker for lipid peroxidation. Lipid peroxidation is the oxidative deterioration of polyunsaturated lipids, which leads to the production of a degraded product called malondialdehyde (Belge et al., 2003). Malondialdehyde is a by-product of lipid peroxidation. Lipid peroxidation has been shown to cause profound alterations in the structural organization and function of cell membranes, including decreased membrane fluidity, increased membrane permeability, inactivation of membrane-bound enzymes and loss of essential fatty acids.

Lipid peroxidation is a complex process which leads to membrane damage and cell death (Belge et al., 2003). Malondialdehyde is formed from metabolism of arachidonic acid to endoperoxides by fatty acid cyclo-oxygenase. These endoperoxides because of their unstable property are rapidly converted into other products, including malondialdehyde. It has been established that increased malondialdehyde values can be due to stress (Belge et al., 2003; Ambali et al., 2010a, b, c; Asala et al., 2011). Draper and Hadley (1990) assert that the generation of malondialdehyde *in vivo* increases with exposure to environmental oxidants. 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., 2010c). Olaifa et al. (2012b) observed an increase in malondialdehyde after subjecting the donkeys to packing. Thus tissue malondialdehyde concentration may be used as biomarker of oxidative stress in donkeys subjected to stress due to packing. This requires further investigation.

Antioxidants as anti-stress agents

Antioxidants are substantial defense network that combat oxidative stress imposed by several stressors, including physical exercise (Asha et al., 2005). Antioxidants provide cells with protection collectively or individually (Ghiselli et al., 2000) against reactive oxygen and nitrogen species. Cells are equipped with an effective and complex antioxidant system, including protective enzymes and biological antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, glutathione, ascorbic acid and vitamin E (Vani et al., 2010). Glutathione peroxidase and catalase remove H_2O_2, while superoxide dismutase catalyzes the dismutation of O_2^- to form H_2O_2 and O_2 (Aruoma, 1998). Natural antioxidants are very important in maintaining high growth levels, reproduction and immunocompetence (Surai, 2006). Several compounds possessing antioxidant properties, which are capable of neutralizing free radicals include fat-soluble (vitamin E and carotenoids) (Ajakaiye et al., 2011) and water-soluble (Ambali et al., 2010a) compounds. Thus antioxidant defense has been considered to be a key element in preventing lipid peroxidation (Henkel, 2011).

ASCORBIC ACID AS AN ANTIOXIDANT AND ANTI-STRESS AGENT

Ascorbic acid is a potent antioxidant compound used to mitigate adverse effects of stress in livestock (Ayo and Sinkalu, 2007), including the donkey (Olaifa et al., 2012b). It has been used as a dietary supplement to pets, even if they are capable of ascorbic acid synthesis (Wang et al., 2001) because ascorbic acid is depleted from the body in stress situations (Hesta et al., 2008). Ascorbic acid, as an antioxidant, detoxifies the harmful reactive oxygen species by giving free molecules of hydrogen, especially when the body's natural antioxidants are exhausted and overwhelmed (Minka and Ayo, 2007b). Ascorbic acid recycling by its regeneration from its oxidized forms such as semidehydroascorbyl radical and dehydroascorbic acid has key functions in maintaining redox homeostasis. It is perhaps the most important water-soluble antioxidant in plasma and plays a fundamental role in the antioxidant defense, both as a scavenger and possibly in the regeneration of other antioxidants (Frei et al., 1989; Urban-Chmeil et al., 2009). It has been observed that ascorbic acid reduces the adverse effects of stress on goats when administered before the commencement of transportation (Minka and Ayo, 2010). Similarly, ascorbic acid has been shown to ameliorate chlorpyrifos-induced erythrocyte fragility (Ambali et al., 2010b), and stress induced by handling, loading, transportation and meteorological factors (Minka et al., 2009; Minka and Ayo, 2010).

Ghanem et al. (2007) also show that ascorbic acid may be used easily by farmers to alleviate water deprivation stress in the semi-arid or arid regions, where scarcity of water is a common challenge imposed by harsh weather and long road transportation. Ascorbic acid as a dietary substance is effective in the amelioration of transportation stress in livestock, including pullets (Minka and Ayo, 2008) and goats (Minka and Ayo, 2011).

Ascorbic acid 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 (Kaminski and Boal, 1992; Harrison and May, 2009).

Ascorbic acid has been shown to induce a lower-frequency fatigue (indicates less muscle damage) (Jakeman and Maxwell, 1993). It has also been demonstrated that ascorbic acid can control the rise of reactive oxidant species, formed during exercise (Shephard et al., 1974; Tauler et al., 2003). If not controlled, reactive oxygen species have the ability to react with cell membranes and damage them, by initiating lipid peroxidation. Kaminski and Boal (1992) examined the relationship between ascorbic acid given to 19 men for three days before exercise (and seven days after) and the muscle damage induced by two bouts of eccentric exercise.

The authors concluded that ascorbic acid reduced muscle damage; however, they did not measure the indices of oxidative stress other than muscle soreness. Supplementation with two 500 g dosages of ascorbic acid for one day is associated with a decreased shift from pre-exercise to post-exercise pro-oxidant activity, when compared to a placebo. The same dosage given over a two-week period did not elicit such great change in peroxidation activity as a one-day supplementation provided (Alessia and Blasi, 1997). This difference may be related to the fact that a one-day dose of ascorbic acid, helped to regenerate other antioxidants in the body such as vitamin E. The authors hypothesize that a two-week period of ascorbic acid supplementation may replenish other antioxidants and then lead to its pro-oxidant properties within the body (Alessia and Blasi, 1997), likely via the Fenton reaction. Thus, there is an obvious lack of research addressing antioxidants and exercise, particularly ascorbic acid. Although the preliminary information on the obtained data seems promising, there is the need for further research in this area.

THE USE OF ASCORBIC ACID IN DONKEYS

Dey et al. (2010) reported a case of heat stress in donkeys and the ameliorative effect of ascorbic acid. Although there is paucity of information in the literature to support the prophylactic use of ascorbic acid in donkeys, its use has been demonstrated in horses, their counterparts (Snow et al., 1987). The Equidae family is known to synthesize ascorbic acid; thus ascorbic acid is a non-essential vitamin. It has been shown that when all animals are subjected to different forms of stressors, there is usually a significant depletion of ascorbic acid. They would, therefore, need to be supplemented with this vitamin in order to ensure balance between oxidants and antioxidants, thereby preventing oxidative stress.

Dey et al. (2010) show that ascorbic acid could serve as a curative agent in donkeys during heat stress. Olaifa et al. (2010a and b) reports that ascorbic acid ameliorated the effect of work stress on rectal temperature and erythrocyte osmotic fragility in donkeys. The uses of antioxidants have not been fully elucidated in donkeys, and there is a need to evaluate its prophylactic use in work stress, especially in those that are subjected to work and adverse environmental conditions.

CONCLUSION

Available information in the literature has shown that using donkeys as pack animals is a cheap and affordable means by which farm produce can be transported in many tropical regions of the world, including Africa. Furthermore, because of the nature of the donkeys' major role in traction and the general view of the people of their 'supposed adaptation', they are often overused and exposed to various stressors, such as work stressors, thermal environment stress and poor management, which eventually can lead to poor performance. Stress experienced in the donkeys may lead to increased production of free radicals. Ascorbic acid, a potent antioxidant, has the ability to ameliorate the effect of free radicals. The possible oxidative effect of packing and the ameliorative role of ascorbic acid in donkeys are yet to be explored. Therefore, it is proposed that more work should be done on the effects of ascorbic acid, and, indeed, other potent antioxidants on work performance of donkeys, reared in the tropical regions of the world, especially in Africa.

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