

Review

Effects of transportation and thermal stress on donkeys in the Northern Guinea Savannah zone of Nigeria: A review

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The paper reviews transportation stress and its negative effects on physiological parameters of livestock, with emphasis on the Guinea Savannah zone of Nigeria. Stress factors acting on animals during road transportation are numerous and the responses of animals to them are complex, non-specific and often detrimental to their health and productivity. In spite of the numerous recommendations and guidelines by many countries on the welfare of animal transport order and their strict compliance by transporters, several studies report severe welfare problems during road transportation of food animals. The impact of the stress on donkeys was also reviewed. It is concluded that measures to alleviate the adverse effects of road transportation stress in livestock will improve their welfare and health.

Key words: Transportation stress, donkeys, stress, welfare, health.

INTRODUCTION

Most donkeys are reared and managed extensively. Donkeys are hardy, docile and intelligent and easily trained draught animals used for farm operations and domestic work in many rural small-holder communities all over the world, especially in the tropics (Bale et al., 2003; Minka and Ayo, 2007b). Historically, the main use of donkeys has been for transport (Fernando and Starkey, 2004). Donkeys can easily be used for fetching water and fire wood. They are not only fast, but can cover long distances, and are tolerant to most diseases (Ngendello and Heemskerk, 2004). In South-Africa, the number of donkeys used for cultivation and transport has increased considerably (Hanekom, 2004). In India, donkeys are used mainly as pack and cart animals for transporting bricks and goods over short distances (Pal et al., 2002). They are of great transportation value as pack animals in areas that modern motorized technology is not available,

especially in mountainous regions with steep and stony paths. Anatomically, donkeys have only five lumbar vertebrae, compared with the six vertebrae in horses (Stecher, 1962), which make them very good pack animals. Donkeys are preferred to other equines because of their affordability, survivability, docile nature and ease of training and handling (Swai and Bwanga, 2008). Donkeys have advantages over oxen for use in rural transport because they are cheaper to purchase, easy to handle and they require short period of training. Besides, they are not very demanding in terms of feed and water requirement (Twerda et al., 1997). Transportation of donkeys to the south-eastern parts of Nigeria for slaughter as source of meat for the population has become a thriving trade in the country (Blench, 2004). In transport-induced stress, free radicals are generated in the body in such a large quantity that the natural antioxidant defense systems

of the body are overwhelmed (Asala et al., 2011). This results in lipoperoxidation of cytomembranes and, consequently, cell damage and destruction (Altan et al., 2003). It has been established that antioxidant supplementation provides beneficial effects against stress-induced tissue damages during transportation of animals by road, although, a preliminary report indicated that the antioxidant, ascorbic acid (AA), also known as vitamin C, may be beneficial in the therapy of heat stress in donkeys (Dey et al., 2010).

MILK AND MEAT OF DONKEYS

The milking of donkeys in Africa is rare and of little economic importance (Blench, 2004). The Western Maasai are reported to milk donkeys, and donkey's milk is used in magical remedies in parts of West Africa. Historically, donkeys were probably not milked because of the labour needed to catch them regularly in low-management systems and the availability of higher yielding alternatives (Blench, 2000). Though donkeys rarely produce excess milk, their milk is very close in composition to that of humans, and, therefore, suited to babies whose mothers have problems in supplying sufficient quality (Jones, 1991).

The extent to which donkeys are eaten is probably greatly under-estimated, since this is something of a taboo for many people. Nonetheless, the wild ass has been hunted to near extinction for its meat (Blench, 2000). In West Africa, the trade in donkeys for meat is essentially of old, sick or exhausted animals that have been used as work animals in the villages of the semi-arid zone. Documentation on consumption of donkeys is poor. In Nigeria, Blench (2004) reported a thriving trade in consumption of donkeys in the south-eastern part of the country. The meat of donkeys is sold locally, and is sometimes more expensive than beef.

MANAGEMENT AND REPRODUCTION OF DONKEYS

Despite the great contribution made by donkeys to daily life of people especially women, they suffer the dual negative impact of low social status and poor management (Swai and Bwanga, 2008). Most of the donkeys are reared under the traditional management system with little or no shelter throughout the year (Minka and Ayo, 2007b). Donkeys possess amazing strength and survive on low-quality food. They tolerate up to 30% dehydration (Swai and Bwanga, 2008).

Fielding (1988) has reviewed the reproductive characteristics of female donkeys world-wide. A working knowledge on the reproduction of donkeys is valuable for its breeding. The donkey is similar in many respects to the horse (Pugh, 2002). The jenny is very similar in many reproduction aspects to the mare. Puberty is usually attained in 1-2 years (Pugh, 2002). In Nigeria, the mean

age of first foaling, 57 months, is substantially higher than in the temperate countries, where about three years is considered usual (Fielding, 1988). Although the estrous cycle has been reported to range from 20 to 40 days, it usually lasts for 23 to 30 days (Fielding, 1988). Estrous usually lasts between 6 and 9 days, with ovulation occurring 5 to 6 days after the onset of estrous (Fielding, 1988). According to Fielding (1988), estrous is characterized by mouth-opening and closing, with salivary dribbling, winking, urinating and tail rising. Gestation length has been reported to be 372 to 374 days (Fielding, 1988). Foal heat usually occurs between 5 and 13 days post-partum. The jack, like the jenny, has many reproduction similarities to the horse (Pugh, 2002). However, some differences do exist. Jack takes longer time to achieve erection and ejaculate and usually takes 5 to 30 min to complete breeding (Pugh, 2002). Complete ejaculation will take 6 to 12 s with a volume of 10 – 80 ml (Gastal et al., 1997).

STRESS AND ITS ADVERSE EFFECTS ON LIVESTOCK

In animal husbandry, stress has usually been imagined as a reflex reaction that occurs unavoidably. Exposure of animals to adverse environmental conditions, consequently, leads to unfavourable states, ranging from discomfort to death (Dantzer and Mormede, 1983). Stress can be defined as a mental or bodily tension produced by external or internal stressors. All animals experience stress as necessary and normal occurrence in their lives (Selye, 1977). However, when stressors become so aversive that an animal is unable to adapt, it enters a state of distress, where its physiology and behaviour become maladaptive. According to Fazio and Ferlazzo (2003), stress, though well-known, is not easy to define. It has been described as the result of adverse effects of environment or management systems, which propel changes in an animal's physiology or behaviour to avoid physiological malfunctioning, thus assisting the animal to cope with its environment. von Borell (2001) defined stress as a condition in an animal that results from the action of one or more stressors that may be of either external or internal origin. Whether a stressor can be classified as harmful depends on the way an organism is able to cope with a threatening situation as it regains a state of homeostasis. Consequently, stress can be measured and monitored in terms of behavioural and physiological alterations that might be pointers of the individual's state of well-being (von Borell, 2001; Fazio and Ferlazzo, 2003; Kashinakunti et al., 2010).

Causes of stress

Stress and distress can be caused by psychological factors (for example, inability to exhibit natural behaviour patterns, fear), physiological factors (physical abnormali-

ties, poor nutrition, pain, pregnancy), environmental factors (including overcrowding, rough-handling, hours of road transportations and excessive heat or cold), physical factors such as poor facility design, vehicle and house (Dantzer and Mormede, 1983).

Adverse effects of stress on animals

When livestock have difficulty in coping with stress, adverse effects of the stress are manifested. This affects their productivity, including weight gain and meat quality. The immune systems of the animals may be compromised, rendering them vulnerable to diseases. Behaviours may become maladaptive, for example excessive aggression; harmful stereotypes or other behaviours that negatively have impact on the animal or its pen mates. The survival rates in young animals may reduce (von Borell, 2001).

Thermoneutral zone

Farm animals have well-established zone of thermal comfort. When a population of homoeothermic animals live in the same surroundings and suffer the same conditions of ambient temperature (AT) and relative humidity (RH), the population adapts itself to this environment by a series of physiological reactions, which tend to reduce heat loss to a minimum in order to ensure constant body temperature (Piccione and Refinetti, 2003). This is defined for such a population as zone of thermal comfort at which the energy losses needed to ensure constant body temperature are minimal, and the animal does not manifest any defensive reactions against cold or heat (Bianca, 1976). Thermoneutral zone is the zone in which physiological defenses against cooling or warming do not involve a notable increase in energy loss in order to maintain body temperature at its normal value (Jean, 1993). Body temperature has a tendency to decrease when the heat losses exceed the heat produced by metabolism. If the losses exceed the lower critical temperature, the body cools (hypothermia) and death occurs due to a cessation of enzyme activity as a result of toxication. When the reverse occurs, the organism warms up, starting at the upper critical temperature, heat loss accelerates and if it cannot stop the heating up, the body temperature rises rapidly (hyperthermia) (Bianca, 1976; Jean, 1993). For donkeys, the environmental requirements are as follows: AT, 23 – 32°C, RH, 30 – 70%, air movement: 0.15 – 0.5 m/s, and ventilation rate, 0.2 – 2.0 m/h/kg body weight (Sainsbury, 1989).

Thermal stress

Thermal stress refers to those meteorological parameters which either interfere with the dissipation of body heat to the environment (high AT and RH) or which impose an external heat load on the animal (solar radiation). The

more heat an animal produces internally by its metabolism, the less its ability to tolerate external heat (Bianca, 1976). Thermal stress is one of the most important stressors in the hot regions of the world (David, 1980; Altan et al., 2003).

A variety of production systems and/or geographical locations results in situations in which animals are exposed to environmental conditions outside their thermoneutral range (West, 2003). According to West (2003), thermal stress is chronic in nature, characterized by intense radiation energy, for an extended period of time, with high RH. Thus, there is often little relief from heat during the evening hours, and intense bursts of combined heat and humidity further depress performance of animals. Reductions in performance of animals during thermal stress can be largely due to elevated AT. These debilitating effects may be further compounded when elevated AT is coupled with solar radiation (Al-Haidary, 2006). When confronted with wide differences in effective AT, livestock compensate for variations in energy flow by altering energy intake, energy loss or energy stored as product. They change rate of performance, the rate at which animals grow, reproduce, or accomplish their desired function, and energetic efficiency of converting feedstuffs to animal product (David, 1980).

Harmattan stress

A wide fluctuation in environmental temperature is one of the main causes of stress in livestock (Minka and Ayo, 2010a). According to Bianca (1976), cold normally represents a smaller problem than heat in livestock. Cold stress results when the AT falls below the lower critical temperature of the animal, the temperature below which an animal has to increase its heat production to prevent its body temperature from falling below the value that is compatible with life (David, 1980).

Three seasons have been described for the Nigeria Guinea Savanna zone: harmattan, hot-dry and hot-humid (Igono et al., 1982; Ayo et al., 1996). The thermal conditions of the environment during these seasons are stressful. Harmattan period lasts from late November to early February in the Nigeria Guinea Savanna zone (Igono et al., 1983). The harmattan season is characterized by high AT (as high as 33°C) in the afternoon hours of the day and relatively low AT (as low as 12°C) in the evening and early morning hours of the day. The season is characterized by cold-dry and dust laden wind (Igono et al., 1983). Animals are observed to shiver during the harmattan season at early morning hours, to increase body heat production so as to maintain the core body temperature within the normal range (Piccione and Refinetti, 2003). Igono et al. (1983) observed a wider range in rectal temperature during the harmattan season than the hot dry season and suggested that the harmattan season is thermally more stressful than the hot-dry season.

Mechanism of stress

Stress is the biological response that animals exhibit in response to stimuli (stressors) which disrupt their homeostasis (Candiani et al., 2008). Stress has widespread effects on physiological systems, including changes in the cardiovascular, endocrine, immune, central nervous and reproductive systems (Obernier and Baldwin, 2006). Mammals respond to stress by releasing a host of primary mediators such as glucocorticoids and catecholamines. These mediators have widespread effects on cells and tissues: they bind to receptors, ion channels and intracellular proteins to cause primary effects, such as activation of signaling cascades and gene expression. Cumulatively, the primary mediators result in secondary outcomes (Lay et al., 1996; Candiani et al., 2008). For example, acute stress causes release of primary mediator adrenaline, which binds to β -adrenergic receptors on the heart, increasing heart rate (Manteca, 1998).

The hypothalamic-adrenal medullary system involves the hypothalamus, pituitary gland, the sympathetic neural pathways to the adrenal medulla, and the release of adrenaline by the adrenal gland. This acute response to stress is referred to as fight-flight syndrome (von Borell, 2001). The hypothalamic-pituitary-adrenocortical (HPA) stress – response systems represents a longer-term, sustained response to stressors and was conceptualized by Hans Selye as general adaptation syndrome (GAS) (Selye, 1946). Selye (1977) classified stress response into three stages: 1. The alarm reaction; 2. stage of resistance; and 3. stage of exhaustion.

The alarm stage is characterized by mobilization of all defense mechanisms in the body to combat adverse effects of stress. If the stress is so severe that continued exposure is incompatible with life, the organism will die within a few hours during this stage. Otherwise, a stage of adaptation or resistance will ensue, since no organism can be maintained continuously in a state of alarm. The adaptive stage is characterized by the vanishing or diminishing of the initial symptoms. After still a more prolonged exposure to the stressor, this acquired adaptation is lost and a third stage of exhaustion is entered into, unless the organism receives emergency aid from some outside source, leads to death (Selye, 1946).

Transportation and its role in animal production

The rising demand in proteins to feed the ever-increasing world population has necessitated the transportation of livestock using different available means of transportation across several ecological zones with different climatic conditions (Minka and Ayo, 2010b). According to Minka and Ayo (2007a), marketing and slaughtering of food animals for meat in abattoirs located outside places where they are produced make transportation unavoidable. Studies on stress and well-being during transportation of livestock have been carried out (Stull and

Rodiek, 2000; Ambore et al., 2009; Minka and Ayo, 2010a; Saeb et al., 2010). The transportation of food animals is an inevitable husbandry practice, which animals unexpectedly encounter, especially those reared predominantly under traditional extensive management systems (Ayo et al., 2006). It is often considered as one of the main causes of stress raising considerable interest, both in economic and animal welfare terms (Saeb et al., 2010). Transportation is a critical phase in animal production and utilization and often considered as one of the main causes of stress (Mormede et al., 1982). The stress reactions overtax the body systems and cause reduction in fitness of the animal by inducing dysfunction of the pituitary, gonadal, adrenal and thyroid glands, and blood composition (Lay et al., 1996; Obernier and Baldwin, 2006). The stress factors acting on animals during transportation are numerous and the responses of the animals to them are complex, non-specific and often detrimental to their health and productivity (Minka and Ayo, 2010b). During transportation, animals are exposed to a number of potential stressors, such as motion of vehicle, noise, vibrations, centrifugal forces, rapidly changing light conditions, heat, cold, poor air quality, mixing of unfamiliar groups, different ages, poor road conditions and the possible lack of water and feed (Fazio and Ferlazzo, 2003; Hartung, 2003). Other stressors acting on the animals include handling, loading, unloading, vehicle type and design, driving methods, vehicle vibration, stocking rate/density and journey duration (Minka and Ayo, 2010b). Transportation has been reported to have adverse effects on meat quality (Fazio and Ferlazzo, 2003). Animal health has been reported to be impaired by various pre-transport and transport conditions (Knowles et al., 1999; Tadich et al., 2005; Minka and Ayo, 2007c).

Transportation of donkeys by road

Few works have been carried out on transportation of donkeys by road, and, there is paucity of information regarding the guidelines on transportation of donkeys. According to Forhead et al. (1995), road transportation is recognized as an environmental stress that can predispose donkeys and ponies to hyperlipdemia. Hyperlipdemia is a severe metabolic crisis more often noticed in donkeys and ponies, particularly obese and pregnant animals, than in large equine breeds (White et al., 1991). Studies of the effects of transport stress have concentrated upon farm animal species (Minka and Ayo, 2007c; Buckham Sporer et al., 2008; Ambore et al., 2009; Nazifi et al., 2009; Ajakaiye et al., 2010; Earley et al., 2010; Ajakaiye et al., 2011b).

Forhead et al. (1995) reported a continuous secretion of cortisol, after transporting donkeys by road for 30 min or four hours. However, in the fed donkeys, these transport-induced changes in the adrenocortical activity were not accompanied by any detectable changes in the metabolites. Stress due to the 4 h journey was not suffi-

sufficiently long or intense for changes in the concentrations of lipids to develop.

Harrington (1989), in donkeys and the law, referred to the Protection of Animal Act, 1911, and the Transit of Animals (Road and Rail) Order 1975 produced guidelines for the transportation of the donkey. The act and the order applied to both horses and donkeys with the aim of reducing their suffering during transportation. It is recommended to stock horse at 1.14 to 1.54 m²/horse during road transportation (Stull, 1999). The vehicle should be constructed and maintained to withstand the action of the weather and the weight of any animal it will carry. The floor space, size and height of the vehicle should be sufficient to make each animal on it comfortable (Harrington, 1989). Feed and water should be made available at intervals not exceeding 12 h, unless the entire journey is completed within the period of 15 h from the time when the journey commenced. The animal should be fed immediately upon its arrival (Harrington, 1989; Hartung, 2003)

Transportation stress factors and stress-induced changes

During transportation, animals are exposed to numerous stress factors and the responses of the animals to them are complex, non-specific and often cause impairment to their health and productivity (Fazio and Ferlazzo, 2003; Minka and Ayo, 2010a; Ajakaiye et al., 2011a, b). Transportation involves several potential stressors, including loading (rough handling) and unloading, deprivation of food and water, poor vehicle design, poor road conditions, extremes of AT and RH, overcrowding, mixing different species and age groups, high air velocity, noise, motion, vibration and length of the journey (Minka and Ayo, 2007a, c, d; Nazifi et al., 2009). The stress factors acting upon domestic animals during their transportation can induce physical and psychic exertions, which disrupt homeostasis, and consequently, the metabolism (Fazio and Ferlazzo, 2003). According to Hartung (2003), the highest physiological and biochemical reactions are observed during loading and unloading and shortly after the start of the journey.

Behavioural changes are often the first and primary sign of distress (Ayo et al., 2007). It has been reported that behavioural indicators of discomfort during transportation are: freezing, back off, attempts to escape, vocalization, kicking and struggling (Broom, 2003). Physiological measurements are also used to assess the level of stress in animals (Fazio and Ferlazzo, 2003) by measuring physiological changes and some haematological and haematological values.

Biomarkers of transportation stress

Stress research indicates that the endocrine, immune and central nervous systems interact and respond to stressful stimuli in a coordinated manner. The presence

of hormones, neurotransmitters and receptors common to all three systems supports the view that communication exists among these systems (von Borell, 2001). Measurements of impaired biological functioning, particularly those connected to increased physiological stress responses, can provide good corroborating evidence of the animal status (Bernabucci et al., 2002; Duncan, 2005). Mammals respond to stress by releasing a host of primary mediators such as glucocorticoids and catecholamines. These mediators have widespread effects on tissues and cells (Obernier and Baldwin, 2006). Non-invasive methods for measuring stress-indicating variables have been developed in addition to classified descriptive behavioural observations, allowing an evaluation of stress by multiple criteria under different conditions and management procedures (von Borell, 2001). Behavioural changes are often the first and primary sign of distress (Lay et al., 1992; Ayo et al., 2002).

Physiological responses to transportation stress

Stress is simply defined as any physiological change in homeostasis. Traditional physiological measurements have relied on quantifying these alterations to homeostasis, including deviations in HR, RR, RT and hormones concentration (Bianca, 1976; Ayo et al., 1998; Ferlazzo, 2003; Obernier and Baldwin, 2006; Ayo et al., 2008). According to Minka and Ayo (2010b), RT, RR and HR are the most relevant on-the-spot diagnostic parameters of the state of an animal's health, before any laboratory analysis is carried out, especially in remote rural areas in the tropics, where modern laboratory facilities may be lacking. The fluctuations in thermal environmental parameters above or below the zone of comfort influences the physiological parameters, which provide accurate information on the adverse effect of stress factor acting on the animal's body (Ayo et al., 2007, 2011; Dzenda et al., 2011). In the work reported by Forhead et al. (1995), HR of donkeys transported by road increased up to 37 beats/minute and RR increased from 22 ± 3 breaths/min to 40 ± 1 breaths/min within 15 min of loading and it remained high during the journey. Minka and Ayo (2007c) recorded increase in the value of RT during the transportation in the hot-dry season. Some works have recorded increase in HR during handling, loading and the commencement of transportation than when the vehicle is in motion or stationary (Broom et al., 1996; Minka and Ayo, 2009).

Haematological parameters and transportation stress

Physiological alterations in animals under stress include changes in blood parameters. Changes in blood picture of animals under stress have been established by many researchers (Stull and Rodiek, 2000; Obernier and Baldwin, 2006; Minka and Ayo, 2007b, 2010a, b; Saeb et

al., 2010) to occur in transported animals. Increase in packed cell volume (PCV), red blood cell count and haemoglobin concentration immediately after loading and pre-transportation has been attributed to the effect of excitement. This leads to splenic contraction and release of erythrocytes into the circulation (Fazio and Ferlazzo, 2003; Saeb et al., 2010). Apparently, changes in the haematological parameters are induced by the increase in circulating catecholamines (Minka and Ayo, 2010b). Haematocrit and total protein concentration are often used as indicators of dehydration in transported horses. An increase in blood glucose, cortisol, albumen, PCV, total plasma proteins have been shown to occur. Neutrophilia and lymphopenia are also evident (Obernier and Baldwin, 2006). Neutrophil:lymphocyte ratio was suggested to be a more reliable marker of stress than cortisol (Stull and Rodiek, 2000). It increases in stressful conditions due to an increase in cortisol secretion (Saeb et al., 2010). The value of these parameters may differ under different conditions, such as the duration of the journey, the method of handling, the age of the animals, and the experience of the animals. These factors need to be put into consideration when evaluating haematological responses to determine stress (Obernier and Baldwin, 2006; Minka and Ayo, 2010b).

Erythrocyte osmotic fragility and transportation stress

Erythrocyte membrane proteins are susceptible to covalent damage, including cross-linking and aggregation by free-radical-induced peroxidation. Extensive peroxidation of lipids causes changes in cell fluidity, a fall in the membrane potential and an increase in the permeability to different ions that finally leads to haemolysis (Ambali et al., 2010; Vani et al., 2010; Bitla et al., 2011). The integrity of the erythrocytes may be determined by evaluating the changes in the erythrocyte osmotic fragility (Asala et al., 2011; Oyewale et al., 2011). During stressful conditions, free radicals are generated. Its continuous production can result in a condition known as oxidative stress (Leeuwenburgh and Heinecke, 2001; Vani et al., 2010). In conditions associated with increased oxidative stress, the antioxidant system is overburdened, resulting in lipoperoxidation damage and subsequent alteration in the composition of the erythrocyte membranes (Altan, et al., 2003; Ambali et al., 2010). This weakens the structural integrity of the erythrocytes, and consequently results in oxidative haemolysis (Leeuwenburgh and Heinecke, 2001; Kataria et al., 2010; Vani et al., 2010). Consequently, haemolysis has been shown to increase in stressful situations in different animals (Ambali et al., 2010; Vani et al., 2010; Asala et al., 2011), including transported pullets (Minka and Ayo, 2008), goats (Minka and Ayo, 2007c, 2009) and pigs (Adenkola et al., 2010; Asala et al., 2011). Oyewale et al. (2011) suggested that the erythrocyte of donkeys is resistant to variation in

temperature and pH of the erythrocyte environment and duration of blood storage, but not as much as that of the camel.

Effects of transportation stress on biochemical parameters

Changes in mineral metabolism triggered off by alterations in the original hormonal status, as a result of environmental stress factors brought about during animal transportation involve chiefly calcium, magnesium, sodium, potassium and chloride (Klaus-Dietrich, 1985, Parker et al., 2003). Stress causes cell stimulation, resulting in an aggressive potential change of cell from rest potential to action potential (Klaus-Dietrich, 1985). There is increase in the concentration of calcium in the interstitial fluids due to increase in catecholamines in a stressful state. White et al. (1991), reported increase in sodium concentration after transportation of horses, indicating increased activity of skeletal and heart muscles. A rise in calcium ion concentration in stressed animals is also manifested by increase in muscle activities (Klaus-Dietrich, 1985). Decrease in potassium concentration observed in animals exposed to stress may be due to the fact that stress induced activation of the hypothalamic-pituitary-adrenal axis and stimulated the secretion of cortisol, resulting in excretion of K^+ (Parker et al., 2003). The changes in the ion milieu give rise to nervous hypersensitive reactions in animals (White et al., 1991; Skull and Rodiek, 2000). Plasma urea is known to increase in transportation stress, indicating an increase in protein and nucleic acid breakdown in the muscles, due to increase in cortisol concentration and prolong food deprivation (Forhead et al., 1995; Knowles et al., 1999). Forhead et al. (1995) reported hyperglycaemia in fasted donkeys, transported for four hours. This may be due to enhanced glucose production and/or impaired utilisation.

Ascorbic acid

Ascorbic acid, also known as vitamin C or L-ascorbic acid or L- ascorbate is an essential nutrient for all livestock. It is important in many metabolic processes, but is not dietary essential in most animals because of their ability to synthesize it naturally (Hesta et al., 2008). Ascorbic acid is a sugar acid with antioxidant properties, its appearance is white to light-yellow crystal or powder and it is water soluble. Vitamin C is an electron donor and, therefore, a reducing agent (Padayatty et al., 2003). In addition to its well-known role as an antioxidant, the vitamin serves as a co-factor in several important enzyme reactions, including those involved in the synthesis of catecholamines, carnitine, cholesterol, amino acids and certain peptide hormones (Harrison and May, 2009).

Biochemistry of vitamin C

Vitamin C (ascorbic acid) is a six-carbon lactone that is

synthesized from glucose in the liver of most mammalian and avian species; for example, donkeys, dogs, goats and chickens, (Harris, 1958; Padayatty et al., 2003). Vitamin C is a reducing agent. All known physiological and biochemical actions of ascorbic acid are due to its action as an electron donor. Ascorbic acid donates two electrons from a double bond between the second and third carbon of the 6-carbon molecules (Harrison and May, 2009). When vitamin C donates electrons, the species formed after the loss of one electron is a free radical, semidehydroascorbic acid or ascorbyl radical. As compared to other free radicals, ascorbyl radical is relatively stable with half-life of 10^{-5} s and is fairly unreactive. This explains why ascorbate may be a preferred antioxidant (Padayatty et al., 2003).

Ascorbic acid is absorbed in the body by both active transport and simple diffusion. Sodium-dependent active transport-sodium-ascorbate co-transporters (SVCTs) and hexose transporters (GLUTs) are the two transporters required for absorption. SVCT1 and SVCT2 import the reduced form of ascorbate across plasma membrane (Balz, 2003). Thus, SVCTs appear to be the predominant system for vitamin C transport in the body. SVCT2 is involved in vitamin transport in almost every tissue, the notable exception being erythrocytes, which lose its SVCT proteins during maturation (Cathcart, 1991). "SVCT2 knock out" animals generally engineered to lack this functional gene die shortly after birth, suggesting that SVCT2-mediated vitamin C transport is necessary for life. With regular intake, the absorption rate varies between 70 and 95%. However, the degree of absorption decreases as intake increases (Balz, 2003). Ascorbate concentrations over renal re-absorption threshold pass freely into the urine and are excreted. Concentrations in the plasma is higher than the threshold concentration are rapidly excreted in the urine with a half-life of about 30 min. Concentrations less than this threshold amount are actively retained by the kidneys, and the excretion half-life for the retained vitamin C increases greatly.

Ascorbate that is not directly excreted in the urine as a result of body saturation or destroyed in other body metabolism is oxidized by L-ascorbate oxidase and removed (Cathcart, 1991; Balz, 2003; Gropper et al., 2004).

Functions of vitamin C

Vitamin C is essential as a highly effective antioxidant, acting to reduce oxidative stress. Also, it an enzyme co-factor for biosynthesis of many important biochemicals (Padayatty et al., 2003; McGregor and Biesalski, 2006).

Vitamin C as an enzyme Co-factor

Vitamin C functions include synthesis of collagen, carnitine and neurotransmitters, the synthesis and catabolism of tyrosine and metabolism of microsome. Vitamin C is an electron donor for different enzymes (Balz, 2003). Three

enzymes participate in collagen hydroxylation. These reactions add hydroxyl group to the amino acid, proline or lysine in the collagen molecule through prolyl hydroxylase and lysyl hydroxylase both requiring vitamin C as a cofactor (Padayatty et al., 2003). Hydroxylation allows the collagen molecule to assume its triple helix structure and making vitamin C essential to the development and maintenance of scar tissue, blood vessels and cartilage (Harrison and May, 2009).

Vitamin C as an antioxidant

Vitamin C is well known for its antioxidant activity (Dey et al., 2010; Hamid et al., 2010; Minka and Ayo, 2010a). When there are more free radicals in the body than antioxidants, oxidative stress results (Altan et al., 2003), which leads to cellular and tissue injuries. Supplementation of antioxidant is needed to quench the excessive free radicals, often generated in the body during stress (Minka and Ayo, 2007c; Valko et al., 2007).

Biological antioxidants play a vital role in protecting cells from exercise-induced oxidative stress. Deficiency or depletion of various antioxidant systems have been shown to exacerbate oxidative tissue injury, whereas supplementation of various antioxidant systems has generated variable results (Banerjee et al., 2003). One of the ways vitamin C performs its antioxidant role is by recycling vitamin E radical back to vitamin E. Vitamin E is an important chain-breaking free-radical scavenger. Its unique location in cellular membrane enhances its efficiency to quench free radicals originating from mitochondrial inner membrane (Banerjee et al., 2003; Minka and Ayo, 2010a). The ameliorating effects of the vitamins are well manifested when the body vitamin C or E is either overwhelmed or exhausted as a result of many stress factors that overtax the animal control systems (Minka and Ayo, 2010a).

Minka and Ayo (2007c) reported no significant difference in the values of neutrophil:lymphocyte ratio, total protein, eosinophils, basophils, monocytes, neutrophils and total leucocytes post-transportation as compared to their corresponding pre-transportation values in goats administered with ascorbic acid and transported by road during the hot-dry season. The administration of ascorbic acid as an antioxidant is known to donate a free molecule of hydrogen that detoxifies the harmful reactive oxygen species generated by the body, especially when the body's natural antioxidants are exhausted or overwhelmed. Ascorbic acid also potentiates gamma-aminobutyric acid which inhibits neurotransmission, and the release of corticosteroid, hence suppressing body temperature (Altan et al., 2003).

Ascorbic acid potentiates gamma-aminobutyric acid, regulates mood and brain function and inhibits the action of cortisol hormones, thereby preventing the release of corticosteroids that are known to affect immunity and

decrease production of eosinophils (Balz, 2003; Minka and Ayo, 2007c; Harrison and May, 2009; Minka and Ayo, 2010b). Ascorbic acid is an outstanding antioxidant that stabilizes free radicals and terminates free-radical induced lipoperoxidation of membranes, thereby maintaining the structural integrity of cells (Adenkola et al., 2009; Asala et al., 2011). The reduction in haemolysis of animals administered with ascorbic acid prior to exposure to stress factors, may be due to the ability of ascorbic acid to protect the integrity of erythrocytes membranes (Adenkola et al., 2010; Alhassan et al., 2010; Asala et al., 2011).

Effects of vitamin C on immune system

Vitamin C is found in high concentration in immune cells, and is consumed quickly during infections. It has been hypothesized that vitamin C modulates the activities of phagocytes, the production of cytokines and lymphocytes, and the number of cell adhesion molecules in monocytes (Heuser and Vojdani, 1997; Balz, 2003; Padayatty et al., 2003). Ascorbic acid stimulates the hexose monophosphate (HMP) shunt and glucose inhibits it. The HMP shunt is a series of chemical reactions that reduce niacin coenzyme NADP to NADPH. Phagocytes need NADPH to create superoxide and other reactive oxygen species that are used to destroy pathogens (Moser, 1987; Hemila, 2003; Walingo, 2005). Upon activation, phagocytes release a set of oxidizing agents intended to kill viruses and bacteria. Many of these oxidants are toxic to the host cells and must be destroyed before they damage the host. Ascorbic acid comes into action as an antioxidant, destroying these excess oxidants (Hemila, 1997; Webb and Villamor, 2007).

Effect of vitamin C as an antihistamine

Vitamin C is a natural antihistamine. It both prevents histamine release and increases the detoxification of histamine (Balz, 2003). According to Chatterjee et al. (1975), other varieties of stress conditions, namely; administration of drugs, vaccines, toxoids and physical stress, the formation or release of histamine was increased in rats and guinea-pigs, and the administration of ascorbic acid resulted in detoxification of histamine in the body.

CONCLUSION

It has been established that antioxidant supplementation provides beneficial effects against stress-induced tissue damages during transportation of animals by road. It is concluded that measures to alleviate the adverse effects of road transportation stress in livestock will improve their welfare and health.

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