

**EFFECTS OF ASCORBIC ACID ON SOME PHYSIOLOGICAL PARAMETERS
AND BIOMARKERS OF OXIDATIVE STRESS IN PACK DONKEYS (*Equus
asinus*) TRANSPORTED BY ROAD DURING THE HARMATTAN SEASON**

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MARCH, 2012

DECLARATION

I declare that the work in the thesis, entitled, “Effects of Ascorbic Acid on Some Physiological Parameters and Biomarkers of Oxidative Stress in Pack Donkeys (*Equus asinus*) transported by Road during the Harmattan Season” has been performed by me in the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria under the supervision of Professor J.O. Ayo, Drs. A. Mohammed and T. Aluwong. The information derived from literature has been duly acknowledged in the text and in the list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

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CERTIFICATION

This thesis, entitled “Effects of Ascorbic Acid on Some Physiological Parameters and Biomarkers of Oxidative Stress in Pack Donkeys (*Equus asinus*) transported by Road during the Harmattan Season” by Ayodele Stephen AKE meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to the Almighty GOD, who enabled me to complete this work.

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I appreciate GOD, for his mercy that was so abundant over my life throughout the course of this work.

To my mother, C. A. Ake, I say thank you. I am also indebted to my amiable mother-in-law GOD bless you.

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ABSTRACT

The aim of the study was to determine the physiological responses of pack donkeys, administered with ascorbic acid (AA) and transported by road for 4 h during the harmattan season in the Northern Guinea Savannah zone of Nigeria. During the study period the thermal environmental parameters, values of rectal temperature (RT), respiratory rate (RR), heart rate (HR), haematological parameters, erythrocyte osmotic fragility (EOF), malondialdehyde and electrolyte concentrations were determined before, during and after the transportation using standard methods. The minimum and maximum values of dry-bulb temperature (DBT) were 13°C and 32 °C, respectively, and the corresponding relative humidity (RH) values of 10 % and 37% were recorded during the study period. The temperature humidity index (THI) fluctuated between 11 °C and 29 °C. The values of thermal environmental parameters were outside the thermoneutral zone, established for the donkey, which indicated that the season was thermally stressful. The overall value of RT (36.6 ± 0.3 °C) obtained in test donkeys and that recorded in the control donkeys (36.3 ± 0.3 °C) did not differ significantly. The RT values recorded in test and control donkeys (35.8 ± 0.2 °C and 35.4 ± 0.2 °C, respectively) for three consecutive days after transportation were not significantly different, but the values were lower ($P < 0.05$) than the corresponding pre-transportation values of 37.0 ± 0.1 °C and 37.0 ± 0.2 °C, respectively. Handling, loading and transportation did not increase the RT above the pre-transportation values and the normal range in the donkey. The RR values of 17.5 ± 1.8 and 17.2 ± 0.9 breaths/min, recorded in tested and control donkeys, respectively after loading were not higher than the corresponding pre-loading values of 16.6 ± 0.8 and 16.1 ± 0.8 breaths/min, respectively. The HR values in test (45.3 ± 2.1 beats/min) and control donkeys (43.8 ± 1.8 beats/min), recorded after the transportation were not significantly different, and they did

not differ from the corresponding pre-transportation values of 48.4 ± 1.6 °C and 49.0 ± 1.2 °C, respectively. The values of N:L ratio obtained in test and control donkeys before transportation were 1.6 ± 0.3 and 1.4 ± 0.5 , respectively; but after transportation, the values decreased to 0.8 ± 0.1 and 1.0 ± 0.1 , respectively. The post-transportation value of N:L ratio was higher ($P < 0.05$) in controls than that of the test donkeys. EOF decreased after loading ($P < 0.05$) at 0.3 % NaCl in the test donkeys compared to that of the control donkeys, and the values obtained were 30.5 ± 0.6 % and 49.5 ± 0.4 %, respectively. At 0.1 % NaCl, the post-transportation EOF value in test donkeys was lower than that of the controls, and the values obtained immediately after the journey and 3 days post-transportation. Malondialdehyde concentration decreased in the test compared to that of the control donkeys after the transportation. Na^+ concentration of 132.8 ± 2.2 mMol/L recorded after transportation in test donkeys was not significantly different from that of 135.6 ± 0.7 mMol/L obtained in control donkeys. There were no significant differences in K^+ , Cl^- , HCO_3^- and urea concentrations obtained pre- and post-transportation in between the control and test donkeys, and the concentrations recorded in both the test and control donkeys after the transportation. In conclusion, AA administration prior to 4 h of road transportation in pack donkeys decreased the adverse effects of the transportation on RT, EOF and N/L ratio, and the ameliorative effect of AA was particularly significant after loading and immediately after the transportation.

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LIST OF ABBREVIATIONS

AA	Ascorbic acid
ANOVA	Analysis of variance
Cl ⁻	Chloride ion
DBT	Dry-bulb temperature
EDTA	Ethylene diamine tetraacetic acid
GAS	General Adaptation Syndrome
GLUTs	Hexose transporters
HMP	Hexose monophosphate
HCO ₃ ⁻	Hydrocarbonate ion
HPA	Hypothalamus-pituitary axis
HR	Heart rate
Kg	Kilogram
MDA	Malondialdehyde
N/L ratio	Neutrophil/Lymphocyte ratio
Na ⁺	Sodium ion
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate-oxidase
NaCl	Sodium chloride
PCV	Packed cell volume
K ⁺	Potassium ion
RH	Relative humidity
ROS	Reactive oxygen specie

RR	Respiratory rate
RT	Rectal temperature
SVCTs	Sodium-ascorbate co- transporters
TBA	Thiobarbituric acid
THI	Temperature humidity index
TP	Total protein

CHAPTER 1

INTRODUCTION

1.0 HISTORY OF DONKEYS

Donkeys (*Equus asinus*) are domestic animals belonging to the equine family which includes horses and Zebras. Asinines (donkeys) share many features with other equidae. The genetic closeness of the equidae family is reflected in the ability to produce viable (non-fertile) offspring such as the mule, hinny and zebrokey. The donkey is one of the oldest domesticated animals of man (Aganga *et al.*, 2003). The early history of the donkey in Africa is difficult to reconstruct because of the sparse archeological data. The wild relatives of the donkey have been limited to virtual extinction, making it difficult to collect the genetic data necessary to establish its ancestry in details. Incidentally, this has depleted the potential gene pool that might be used to revitalize domestic breed (Blench, 2000). According to Blench (2000), the precise wild progenitor of the domestic donkey is disputable. The new findings suggest that two populations of wild asses in Africa were the first to be domesticated by people of thousands years ago, and these donkeys then travelled with people to other parts of the world (Aganga *et al.*, 2000; Blench, 2004). The African wild ass is made up of two separate species, the Nubian wild ass (*Equus asinus africanus*) and the Somalian or Abyssinian wild ass (*Equus asinus somaliensis*). The Nubian wild ass is the progenitor of the domestic donkey, although other races may also have contributed to the gene-pool (Blench, 2000).

Donkeys are kept in Africa for work, breeding, milk and meat. Of these, work is reported to be the most important, followed by meat (Blench, 2000). Recently the use of donkeys for packing firewood, coal and cow dung which serve as an alternative source of domestic

energy has increased greatly because of the irregular supply of energy for domestic cooking in some parts of Asia and Africa (Pal *et al.*, 2002; Minka and Ayo, 2007b). Donkeys play important roles in the movement of people and agricultural goods in Nigeria and several other African countries (Hassan, 1995; Bale *et al.*, 2003). According to Minka and Ayo (2007b), donkeys are particularly useful in those areas with hilly and rocky terrain and in exclusively dry environments, which are inaccessible to motorized forms of transport.

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, 2000). Transportation of animals outside of the places where they are been produced is inevitable (Minka and Ayo, 2010a).

1.2 STATEMENT OF THE PROBLEM

Donkeys are of great socio-economic importance in rural settings in northern Nigeria and other African countries (Bale *et al.*, 2003). It is estimated that there are over 3 million donkeys in Nigeria with the northern parts of the country having the highest number. In Nigeria, this species of animal has been virtually neglected by researchers, government, livestock-policy makers, veterinarians and livestock entrepreneurs, yet they are much a part of the rural and urban communities in the northern Nigeria (Hassan, 1995; Lopez and Morales, 2001; Bale *et al.*, 2003). Donkeys play a major role in the provision of rural and urban transport and other agricultural draft purposes. Inadequate information on its physiology, health and welfare requirement made it difficult to promote them as working animals in the tropics (Pearson *et al.*, 1998; Minka and Ayo, 2007b).

Transportation is often considered as one of the main causes of stress. Road transportation is a critical phase in animal production and utilization (Minka and Ayo, 2010b; Saeb *et al.*, 2010). The physical and psychic exertion occurring during transport of food animals disrupt their homeostasis and metabolism and as a result of the exertion, road transport stress increases activity of enzymes and hormones (Ayo and Oladele, 1996; Minka and Ayo, 2010b). Animal health can be impaired by various pre-load and transport conditions. These conditions may cause, injury, reduce performance, cause increased morbidity and mortality rate and consequently substantial economic losses due to loss of live weight and poor meat quality (Minka and Ayo, 2010a).

Physiological, including hematological, behavioral and biochemical parameters are useful diagnostic tools in the practice of veterinary medicine (Pritchard *et al.*, 2006; Lemma and Moges, 2009). Haematological and biochemical parameters are good indicators of the physiological status of animals under different conditions (Ambore *et al.*, 2009). There is paucity of knowledge on haematological and biochemical changes in donkeys during stress induced by road transportation. The parameters have been established to be of great value in the measurement of potential biomarkers of stress (Salar-Amoli *et al.*, 2009; Nazifi *et al.*, 2009). Many researchers have employed haematological parameters in the evaluation of adverse effects of stress on livestock (Mushi *et al.*, 2000; Minka and Ayo, 2007a; Ambore *et al.*, 2009; Bitla *et al.*, 2011). Haematological and biochemical parameters have been shown to be affected by seasonal variations (Kiem *et al.*, 2002; Piccione and Caola, 2002). The parameters are of great value in the measurement of potential biomarkers of stress (Adenkola *et al.*, 2009; Nazifi *et al.*, 2009; Salar-Amoli *et al.*, 2009; Minka and Ayo, 2010a; Bitla *et al.*, 2011).

The extreme in environmental temperatures can adversely affect the health, reproduction and performance of equids (Dey *et al.*, 2010), transported during the different seasons of the year. Donkeys are often transported from the northern to the southern part of Nigeria for slaughter (Blench, 2000). The harmattan season is characterized by high ambient temperature in the afternoon hours of the day and relatively low ambient temperature in the evening and early morning hours of the day. The season is characterized by cold-dry and dust laden wind (Igono *et al.*, 1982). Thus, the harmattan season is associated with both cold and heat stress.

In transport induced-stress, free radicals are generated in the body in such a large quantity that the natural antioxidant defence systems of the body are overwhelmed. This results into lipoperoxidation of cytomembranes and consequently, cell damage and destruction (Cooper *et al.*, 2002; 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 (Minka and Ayo 2007a, c, d; Valko *et al.*, 2007; Ajkaiye *et al.*, 2010; Minka and Ayo, 2010a, b). Although some preliminary report has indicated that the antioxidant, ascorbic acid or vitamin C may be beneficial in the therapy of heat stress in donkeys (Dey *et al.*, 2010), there is paucity of information on the effects of antioxidants in the amelioration of stress induced by road transportation in pack donkeys.

1.3. JUSTIFICATION OF THE STUDY

Of all domesticated animals, the donkey is the least studied, despite the critical role that it plays in transport throughout human history (Aganga *et al.*, 2003; Ayele *et al.*, 2006).

Donkeys are able to survive in extreme conditions, but mortality associated with heat stress

in donkeys has been reported in India (Dey *et al.*, 2010). The effects of antioxidants in managing thermal stress in donkeys need to be investigated. Available information in the literature indicates the potential role of ascorbic acid as an antioxidant in ameliorating adverse effects of environmental stress (Ayo *et al.*, 2007; Minka and Ayo, 2007c; Adenkola *et al.*, 2009; Harrison and May, 2009). There is the need to obtain physiological and biochemical reference values for donkeys in Nigeria, especially during the harmattan season (Jordana *et al.*, 1998; Mushi *et al.*, 2000), which have been lacking in the available literature. This is because for years to come the donkey will continue to play vital roles in the rural and urban settlements, especially in areas with bad terrain, hilly and rugged landscape with no motorable forms of transportation (Bale *et al.*, 2003).

1.4. GENERAL AIM OF THE STUDY

The general aim of the study is to investigate the effects of ascorbic acid on some physiological parameters and biomarkers of oxidative stress of pack donkeys (*Equus asinus*), transported by road during the harmattan season.

1.5. SPECIFIC OBJECTIVES OF THE STUDY

The specific objectives of the study were to determine:

- i. The physiological responses of pack donkeys to road transportation.
- ii. Biomarkers of oxidative stress in pack donkeys, transported by road.
- iii. The effects of ascorbic acid on physiological responses and biomarkers of oxidative stress in pack donkeys, transported by road during the harmattan season.

1.6. RESEARCH HYPOTHESES

Ho. 1 Road transportation does not have any effect on physiological parameters and biomarkers of oxidative stress in pack donkeys during the harmattan season.

Ho. 2. Ascorbic acid does not modulate the adverse effects of road transportation on physiological responses and biomarkers of oxidative stress in pack donkeys during the harmattan season.

CHAPTER 2

LITERATURE REVIEW

2.1 DONKEYS AND THEIR USES

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).

2.1.1 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.

2.1.2 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 worldwide. A working knowledge 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 23 – 30 days (Fielding, 1988). Estrous usually lasts between 6 and 9 days, with ovulation occurring 5 - 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 – 374 days (Fielding, 1988). Foal heat usually occurs between 5 and 13 days post-partum. The jack, like the jenny, has many reproductive similarities to the horse (Pugh, 2002). However, some differences do exist. Jack takes longer time to achieve erection and ejaculate and usually takes 5 – 30 minutes to complete breeding (Pugh, 2002). Complete ejaculation will take 6-12 seconds with a volume of 10 – 80 ml (Gastal *et al.*, 1997).

2.2 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).

2.2.1 Causes of Stress

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

2.2.2 Adverse Effects of Stress on Animals

When livestock have difficulty coping with stress adverse effects of the stress are manifested. Thus, 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 impact the animal or its pen mates. The survival rates in young animals may reduce (von Borell, 2001).

2.2.3 Thermoneutral Zone

Farm animals have well-established zone of thermal comfort. When a population of homoeothermic animals live in the same surroundings and suffers 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 defences 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 – 32⁰C, 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).

2.2.4 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 for tolerating 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 of 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)

2.2.5 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.

2.2.6 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 cell 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 epinephrine 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. the stage of resistance, and 3. the stage of exhaustion.

The alarm stage is characterized by mobilisation 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 which, unless the organism, receives emergency aid from some outside source, leads to death (Selye, 1946).

2.3.0 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; Minka and Ayo, 2007a). 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).

2.3.1 Transportation of Donkeys by Road

Not much work has 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 hyperlipaemia. Hyperlipaemia 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 minutes 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 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 produce 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 – 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).

2.3.2 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 deleterious 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 haematochemical and haematological values.

2.3.3 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).

2.4.0 PHYSIOLOGICAL RESPONSES TO TRANSPORTATION STRESS

Stress simply defined as any physiological change in homeostasis. Traditional physiological measurements have relied on quantifying these alterations to homeostasis, such deviations in HR, RR, RT and hormones concentrations (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 provides accurate information on the adverse effect of stress factor acting on the animal's body (Ayo *et al.*, 2007; Ayo *et al.*, 2011; Dzenda *et al.*, 2011). In the work reported by Plyaschenko and Sidorov (1987), 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 minutes 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. Many 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).

2.5.0 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, 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).

2.6.0 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 cells erythrocyte osmotic fragility (Asala *et al.*, 2011; Oyewale *et al.*, 2011). During stressful conditions, free radicals are generated. Its continuous production can result to 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 camel.

2.7.0 EFFECTS OF TRANSPORTATION STRESS ON BIOCHEMICAL PARAMETERS

Changes in mineral metabolism triggered off by alterations in the original hormonal status, as result of environmental stress factors brought about during animal transportation involves 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 increase activity of skeletal and heart muscles. A rise in calcium ion concentration in stressed animals is also manifested by increase 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.

2.8.0 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).

2.8.1 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 donkey, dog, goat, chicken, (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} seconds and is fairly unreactive. This explains why ascorbate may be a preferred antioxidant (Padayatty *et al.*, 2003).

2.8.2 Absorption, Transport and Elimination of Vitamin C

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 – 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 higher than the threshold concentration are rapidly excreted in the urine with a half-life of about 30 minutes. 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).

2.8.3 Functions of Vitamin C

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

2.8.3.1 *Vitamin C as an Enzyme Cofactor*

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 acids 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).

2.8.3.2 *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 (N:L) ratio, total protein (TP), eosinophils, basophil, monocytes, neutrophils and leucocytes post-transportation 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 (ROS) generated by the body, especially when the body's natural antioxidants are exhausted or overwhelmed. Also, ascorbic acid potentiates γ -aminobutyric acid which inhibits neurotransmission, and the release of corticosteroid, hence suppressing body temperature (Altan *et al.*, 2003).

Ascorbic acid potentiates γ -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 as an outstanding antioxidant 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 erythrocytes 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).

2.8.3.3 *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 is a series of chemical reactions that reduces 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 do damage to the host. Ascorbic acid comes into play as an antioxidant, destroying these excess oxidants (Hemila, 1997; Webb and Villamor, 2007).

2.8.3.4 *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), under variety of stress conditions namely administration of drugs, vaccines, toxoids and physical stress, the formation or release of histamine was increased in rat and guinea-pig, and the administration of ascorbic acid resulted in detoxification of histamine in the body.

CHAPTER 3

MATERIALS AND METHODS

3.1 EXPERIMENTAL SITE

The study was conducted during the harmattan season from 3rd-13th January, 2011 at the Research Pen of the Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria (11°10'N, 7°38'E), Kaduna State, located in the Northern Guinea Savannah zone of Nigeria.

3.2 EXPERIMENTAL ANIMALS AND MANAGEMENT

Thirteen Nubian, apparently, healthy pack donkeys of both sexes, aged between two to four years and weighing between 70 – 137 kg was purchased from donkey market at Seme (11° 10'N, 7° 02'E) in Faskari Local Government Area of Katsina State served as subjects for the experiment. They were housed in the Research Pen of the Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

The donkeys were pre-conditioned for a period of two weeks before the commencement of the experiment, during which they were screened for haemoparasitic, helminthic and ectoparasite infestations. Faecal and blood samples were collected and sent to Helminthology Laboratory and Protozoology Laboratory, respectively. Blood samples were also sent to Clinical Pathology Laboratory for full blood counts. The result from Protozoology Laboratory showed no parasite, Helminthology Laboratory reported *Strongyle* egg (++) , while Clinical Pathology Laboratory revealed normal blood picture. The donkeys were dewormed with albendazole at the dose rate of 2.5 mg/kg body weight, and administered with oxytetraciline long-acting at the dose rate of 20 mg/kg body weight

prophylactically. The donkeys were fed on straws and legumes at the ratio of 4:1, supplemented with wheat bran. Mineral salt-lick was also provided, and water was given twice per day (morning and evening) throughout the study period (Aganga *et al.*, 2000; Mengistu *et al.*, 2005; Pearson, 2005).

3.3 EXPERIMENTAL DESIGN

The donkeys were divided into two groups. Group I, served as test animals and consisted of eight donkeys; four males, and four females. The donkeys were administered with ascorbic acid orally on the day of transportation at the dose rate of 300 mg/kg (Chervyakov *et al.*, 1977) 15 minutes before loading into the vehicle. Group II which served as control consisted of five donkeys, three males and two females. Food and water were withdrawn 12 h before and throughout the transportation period. The vehicle travelled at a speed of 40 – 50 km/h along Zaria - Funtua road from Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, on tarred, smooth and rough road, covering a total distance of 140 km.

3.4 VEHICLE DESIGN AND LOADING

A standard Mitsubishi truck (made in Japan) was used for the journey. The transportation process was carried out according to the guidelines governing welfare of animals, transported by road (Knowles *et al.*, 1993). The floor of the vehicle, which measured 4.2 m x 1.9 m, was covered with wood shavings. The height of the vehicle measured 1.2 m. The donkeys were loaded individually by three people. In a relatively calm condition, two persons easily caught one donkey at a time randomly and calmly led it to the third person already inside the vehicle who in turn tethered the donkey to the metal rod of the vehicle's

body using a 1 m sisal rope that was tied to a neck loop. The donkeys were stocked at a density rate of 1.0 m² per animal (Stull, 1999).

3.5 THERMAL ENVIRONMENTAL PARAMETERS

The dry-bulb temperature and relative humidity were measured using a dry- and wet-bulb thermometer (Brannan^R, England) at the experimental site at 06:00 h, 13:00 h and 18:00 h for three consecutive days before and after transportation, and on day seven after transportation. The parameters were also recorded immediately after loading, during the journey hourly, immediately (15 minutes) after unloading and three hours after transportation (Minka and Ayo, 2007c).

3.6 PHYSIOLOGICAL PARAMETERS

The RT, RR and HR of the donkeys were recorded concurrently with the thermal environmental parameters for three consecutive days at 06:00 h, 13:00 h and 18:00 h before the day of transportation to establish the base-line values. On the day of transportation, the parameters were also recorded immediately (15 minutes) after loading, during the journey (one hour, two hours after the commencement of the journey), immediately after unloading and three hours post-transportation. The recordings at 6:00 h, 13:00 h and 18:00 h were done for three consecutive days and on days 3 and 7 post-transportation. The RT was measured using digital thermometer (Electron thermometer, COCET^R, Kangful, Zhejiang Yueging, China), which was inserted about 3.5 cm into the rectum until an alarming sound was heard, indicating the end of the reading, usually after about 50 seconds. RR was recorded by counting the number of respiratory flank movements for one minute, and the HR was measured through auscultation of the heart by placing a stethoscope (Sprague

Rappaport Type Stethoscope, England) between the 4th and 5th rib on the left side of the animal at the level of the intercostal muscles and counting the number of heart beats per minute (Minka and Ayo, 2007b; Ayo *et al.*, 2008).

3.7 BLOOD SAMPLE COLLECTION AND ANALYSIS

Blood was collected a day before transportation, immediately (15 min) after loading, immediately (15 min) after transportation, and on days three and seven after transportation. Eight ml of blood sample was collected following light and calm restraint from each donkey by jugular venipuncture using a sterile 18G needle, fitted into a 10 ml sterile syringe. The blood was immediately emptied into two 5 ml sterile sample bottles. One sample bottle contained ethylene diamine tetra-acetic acid (EDTA) as an anticoagulant, while the other bottle did not. The blood samples with anticoagulant were used for haematological analysis and erythrocyte osmotic fragility test, while serum was obtained from blood sample in plain bottle and was used for biochemical analysis and serum malondialdehyde test.

3.7.1 Haematological Analysis

Two blood smears were prepared on microscope slides. They were dried at room temperature and stained with Wright's Giemsa (Bayer, Diagnostic Division, Elkhart, USA), using an automatic slide strainer. Differential leucocyte count was carried out under the microscope oil immersion objective (x 100) and 100 cells per slide were counted using the straight-edge method as described by Schalm *et al.* (1975). Whole blood packed cell volume was determined directly after sampling using a microhaematocrit centrifuge and reader (Hawksley, West Sussex, U.K.). Haemoglobin concentration was determined by the

met-haemoglobin technique, while total protein concentration was determined from blood plasma as described by Knowles *et al.* (1993).

3.7.2 Evaluation of Erythrocyte Osmotic Fragility

Erythrocyte osmotic fragility was determined using the method described by Faulkner and King (1970) as modified by Oyewale (2011). Briefly, 10 µml of blood sample collected from each donkey was pipetted into each of the test tubes, containing increasing concentrations (0.0, 0.1, 0.3, 0.5, 0.7, 0.9%) of NaCl (pH of 7.4), and then followed by careful mixing and incubation for 30 minutes at room temperature. The test tubes were centrifuged at 2000 x g for 10 minutes using a centrifuge model IEC HN-SII (Damon Division, Canada, U.K.). The supernatant was transferred into a glass cuvette and the absorbance of the supernatant measured colorimetrically with Spectronic 20 (Bausch and Lomb, Lexington, USA) at the wavelength of 540 nm. The percentage haemolysis for each sample was calculated using the following formula:

$$\text{Percentage (\%) Haemolysis} = \frac{\text{Optical Density of Test Solution}}{\text{Optical Density of Standard Solution}} \times 100$$

3.7.3 Biochemical Analysis

The blood in the plain vacutainer was allowed to clot. Serum was obtained after centrifuging at 2000 × g for 10 minutes. The serum was analysed for urea-nitrogen and electrolytes (Cl⁻, Na⁺, K⁺ and HCO₃⁻). All the parameters were determined using an auto-analyzer (Bayer Express Plus, Bayer, Dusseldorf, Germany).

3.7.4. Evaluation of Erythrocyte Malondialdehyde Concentration.

Erythrocyte malondialdehyde (MDA) concentration as a maker of lipid peroxidation was determined by the double-heating method of Draper and Hadley (1990) as modified by Altuntas *et al.* (2002). The principle of the method is based on spectrophotometric measurement of the colour produced during the reaction of thiobarbituric acid (TBA) with MDA. Briefly, 2.5 ml of 100 g/l trichloroacetic acid was added to 0.5 ml of erythrocytes in a centrifuge tube and placed in a boiling water bath for 15 min. After cooling in tap water, the mixture was centrifuged at $1000 \times g$ for 10 min, and 2 ml of the supernatant was added to 1 ml of 6.7 g/l TBA solution in a test tube and placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance measured using a UV spectrophotometer (Jenway, 6405 model, Apel, Japan) at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex $1.56 \times 10^5 \text{ cm}^{-1} \text{ m}^{-1}$, and expressed in nanomoles per gram of haemoglobin. The haemoglobin concentration was determined using the method of Dacie and Lewis (1991).

3.8 STATISTICAL ANALYSIS

Data obtained were expressed as mean \pm standard error of the mean (mean \pm SEM) and subjected to one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison *post-hoc* test, using Graphpad Prism version 4.0 for Windows (GraphPad Software, San Diego, U.S.A.) to compare differences between the mean, obtained at the different times of recording in the test groups. Pearson's correlation analysis was used to evaluate the relationship between the thermal environmental and physiological parameters. Values of $P < 0.05$ were considered significant.

CHAPTER 4

RESULTS

4.1 THERMAL ENVIRONMENTAL PARAMETERS DURING THE STUDY PERIOD

Thermal environmental parameters during the study period are presented in Table 4.1. The mean values of DBT, RH and THI recorded at the experimental site three days before the journey were $22.8 \pm 2.5^{\circ}\text{C}$, $26.7 \pm 3.0\%$ and $21.2 \pm 2.5^{\circ}\text{C}$, respectively. The values of the parameters obtained after loading were 24.0°C , 26% and 23.0°C , respectively and they were not significantly different from the corresponding values recorded before the journey.

The AT, RH and THI values recorded inside the vehicle during 4-h road transportation are shown in Table 4.1. The mean AT and THI values of $27.3 \pm 2.9^{\circ}\text{C}$ and $25.7 \pm 2.4^{\circ}\text{C}$, respectively were higher than their corresponding values after loading, while the mean RH value of $20.7 \pm 6.0\%$ was lower ($P < 0.05$) compared to that recorded after loading. The AT, RH and THI values obtained three hours after the journey, when compared with the corresponding values recorded 3 days after the journey did not differ. The thermal environmental values recorded 3 days and 7 days after the journey were not significantly different.

4.2 RECTAL TEMPERATURE, RESPIRATORY AND HEART RATES OF DONKEYS BEFORE TRANSPORTATION

The RT values of the test and control donkeys three days before the transportation are shown in Figure 4.1. The overall RT values of $37.0 \pm 0.1^{\circ}\text{C}$ and $37.0 \pm 0.2^{\circ}\text{C}$ recorded in the tested and control donkeys, respectively prior to the transportation were not different.

The RR values of the test and control donkeys obtained three days before the transportation are shown in Figure 4.2. The RR values of 16.6 ± 0.8 breaths/minute and 16.1 ± 0.4 breaths/minute recorded in test and control donkeys respectively were not significantly different. The HR values in the test and control donkeys recorded three days before transportation were not significantly different.

Table 4.1: Thermal environmental parameters during the study period

Period	Ambient temperature (°C)	Relative Humidity (%)	Temperature humidity-index (°C)
Before journey (Mean ± SEM)	22.8 ± 2.5 ^a (13 – 32)	26.7 ± 3.0 ^b (10 – 37)	21.2 ± 2.5 ^a (11 – 29)
After loading	24	26	23
During journey (Mean ± SEM)	27.3 ± 2.9 ^a (22 – 32)	20.7 ± 6.0 ^b (10 – 31)	25.7 ± 2.4 ^a (21 – 29)
3 Hours after journey	20	17	19
3 Days after journey (Mean ± SEM)	20.2 ± 1.7 ^a (13 – 28)	21.2 ± 1.6 ^a (11 – 27)	18.9 ± 1.9 ^a (11 – 27)
7 Days after journey (Mean ± SEM)	21.7 ± 4.4 ^a (13 – 27)	20.7 ± 2.8 ^a (15 – 24)	20.3 ± 4.7 ^a (11 – 26)

^{a, b, c}=Values with different superscript letters are significantly (P < 0.05) different.

Values in parenthesis indicate the ranges

4.3 EFFECTS OF HANDLING AND LOADING ON THE RECTAL TEMPERATURE AND RESPIRATORY AND HEART RATES OF TESTED AND CONTROL DONKEYS

The effects of handling, loading and AA on RT, RR and HR of donkeys are shown in Figures 4.1, 4.2 and 4.3, respectively. The RT values of $36.0 \pm 0.3^{\circ}\text{C}$ and $35.7 \pm 0.5^{\circ}\text{C}$ obtained in the test and control donkeys after loading respectively, were lower than the pre-loading values. The RR values of 17.5 ± 1.8 breaths/minute and 17.2 ± 0.9 breaths/minute obtained in test and control donkeys after loading were higher ($P < 0.05$) than the corresponding pre-loading values. HR values of 42.0 ± 3.0 beats/minute and 48.8 ± 7.2 beats/minute were not significantly different from the corresponding pre-loading values, although HR value in the test donkeys was lower compared to that of the control donkeys.

The RT changes recorded during four-hours of road transportation in test donkeys rose ($P < 0.05$) from $36.0 \pm 0.3^{\circ}\text{C}$ post-loading to $36.6 \pm 0.2^{\circ}\text{C}$ during the first hour of the journey. Thereafter, the value increased to $37.1 \pm 0.1^{\circ}\text{C}$, and at the 4 h of transportation the RT value recorded was $37.8 \pm 0.1^{\circ}\text{C}$. In the control donkeys, the RT recorded post-loading was $35.5 \pm 0.2^{\circ}\text{C}$ at the first hour of the journey, it rose to $36.3 \pm 0.1^{\circ}\text{C}$ and $37.7 \pm 0.1^{\circ}\text{C}$ during the 2nd and 4th hour of the journey, respectively. The overall value of RT obtained in test donkeys was significantly ($P < 0.05$) higher than the value recorded in the control donkey. Three hours after the journey, the RT values recorded in the test and control donkeys were not significantly different. Similarly, the RR and HR values obtained in the test and control donkeys three hours after journey period were not significantly different. (Figures 4.2 and 4.3).

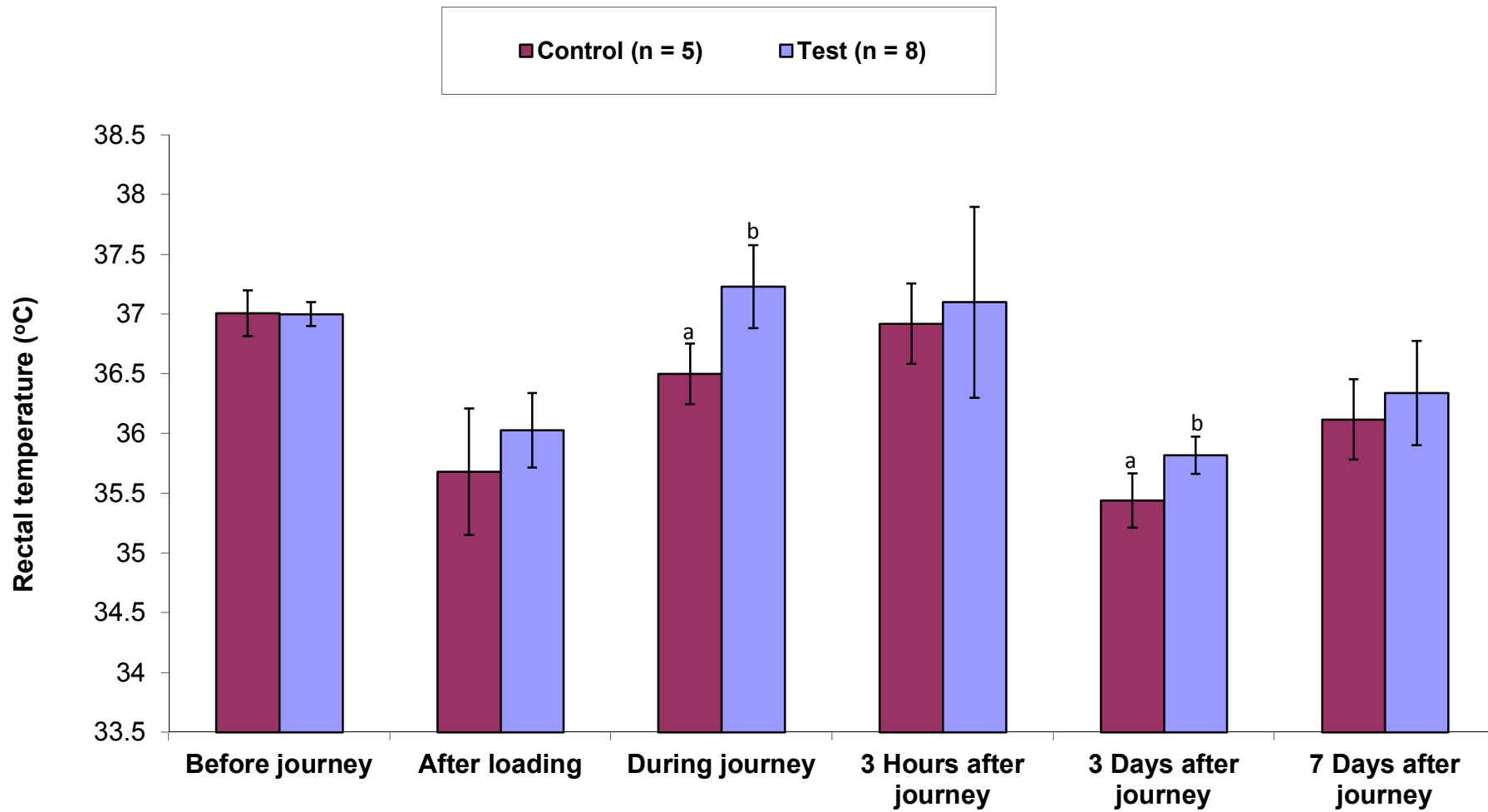


Figure 4.1: Rectal temperature of donkeys transported by road during the harmattan season

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

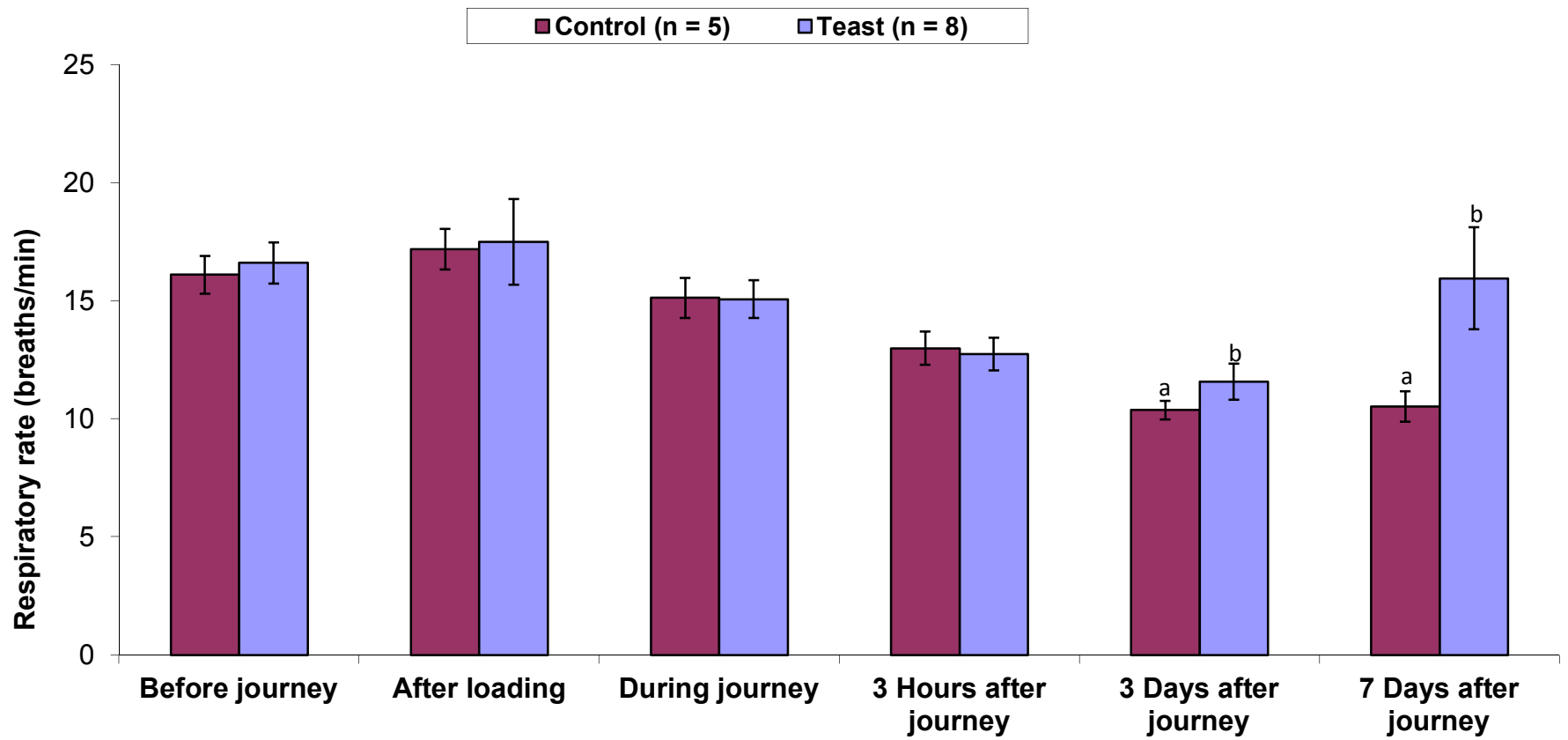


Figure 4.2: Respiratory rate of donkeys transported by road during the harmattan season

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

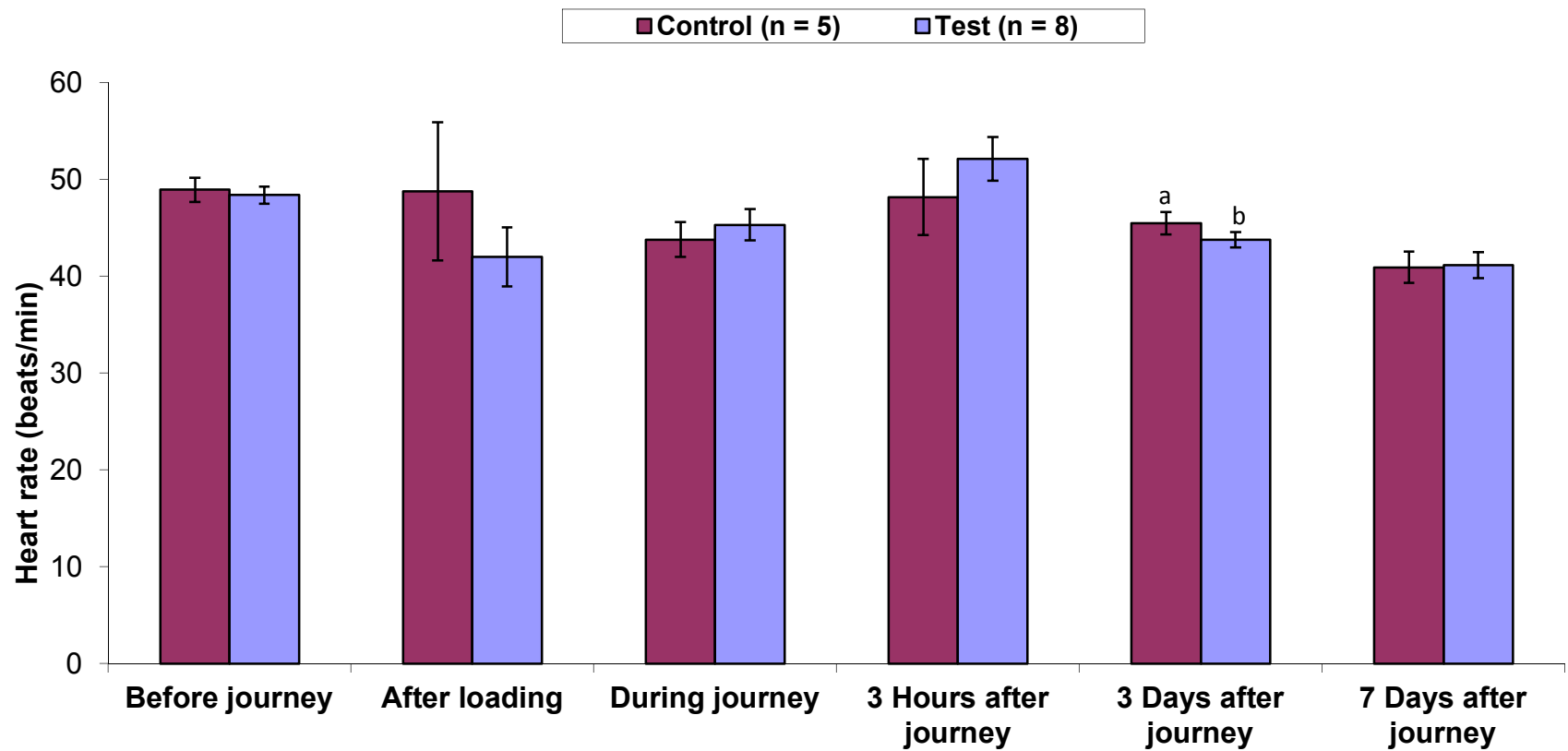


Figure 4.3: Heart rate of donkeys transported by road during the harmattan season

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

The fluctuations in RT values of test and control donkeys obtained during the first 3 days and on day 7 after transportation are shown in Figure 4.1. The RT values recorded in both test and control donkeys for three consecutive days after the transportation were significantly ($P < 0.05$) different and the values were lower ($P < 0.05$) than the pre-transportation values. The RT values recorded on day 7 were higher ($P < 0.05$) compared to those recorded 3 days after the transportation in both the test and control donkeys. The RR and HR values of test and control donkeys 3 days and on day 7 after transportation are shown in Figures 4.2 and 4.3, respectively. The RR values in test and control donkeys were significantly ($P < 0.05$) different both on 3 days and day 7 after the journey. The values of HR in test and control donkey were not significantly different.

4.4 RELATIONSHIPS BETWEEN THERMAL ENVIRONMENTAL AND PHYSIOLOGICAL PARAMETERS OF DONKEYS BEFORE, DURING AND AFTER ROAD TRANSPORTATION

Table 4.2 shows the relationship between the thermal parameters and physiological parameters before four hours of road transportation of test and control donkeys. The relationship between AT and physiological parameters were more significant ($P < 0.001$), especially in control donkeys. The correlation between the RH and the physiological parameters was not significant. Table 4.3 shows the relationship between the AT, RH, THI, RT, RR and HR recorded during the transportation. The values of the thermal environment parameters in the vehicle during the transportation were significantly higher ($P < 0.05$) than the corresponding values obtained at the experimental site before the transportation.

Table 4.2: Relationships between thermal environmental conditions and physiological parameters of donkeys before four hours of road transportation during the harmattan season.

Correlated parameters	Control (n = 5)	Test (n = 8)
Ambient temperature and rectal temperature	0.7489 ^{**}	0.6004 [*]
Ambient temperature and respiratory rate	0.8134 ^{***}	0.7418 ^{***}
Ambient temperature and heart rate	0.8092 ^{***}	0.8748 ^{***}
Relative humidity and rectal temperature	0.3875 ^{NS}	0.4613 ^{NS}
Relative humidity and respiratory rate	-0.3800 ^{NS}	-0.3050 ^{NS}
Relative humidity and heart rate	0.08427 ^{NS}	0.2167 ^{NS}
Temperature humidity index and rectal temperature	0.7866 ^{**}	0.6430 [*]
Temperature humidity index and respiratory rate	0.7739 ^{**}	0.7562 ^{**}
Temperature humidity index and heart rate	0.7170 ^{**}	0.8785 ^{***}

^{NS} = non-significant correlation (P > 0.05), ^{*} P < 0.05, ^{**} P < 0.01, ^{***} P < 0.001.

Table 4.3: Relationships between thermal environmental conditions and physiological parameters of donkeys during four hours of road transportation in the harmattan season.

Correlated parameters	Control (n = 5)	test (n = 8)
Ambient temperature and rectal temperature	-0.8654 ^{***}	-0.7959 ^{**}
Ambient temperature and respiratory rate	0.7028 ^{***}	0.2414 ^{NS}
Ambient temperature and heart rate	-0.7018 ^{***}	-0.8214 ^{***}
Relative humidity and rectal temperature	-0.4612 ^{NS}	-0.4973 ^{NS}
Relative humidity and respiratory rate	0.8415 ^{***}	0.9706 ^{***}
Relative humidity and heart rate	0.2070 ^{NS}	0.3693 ^{NS}
Temperature humidity index and rectal temperature	-0.6035 [*]	-0.2283 ^{NS}
Temperature humidity index and respiratory rate	0.8122 ^{***}	0.1650 ^{NS}
Temperature humidity index and respiratory rate	-0.5444 [*]	-0.6681 ^{**}

^{NS} = non-significant correlation (P > 0.05), ^{*} P < 0.05, ^{**} P < 0.01, ^{***} P < 0.001

Table 4.4: Relationships between thermal environmental conditions and physiological parameters of donkeys after four hours of road transportation during the harmattan season.

Correlated parameters	Control (n = 5)	Test (n = 8)
Ambient temperature and rectal temperature	0.6388*	0.5937*
Ambient temperature and respiratory rate	0.8530***	0.7871***
Ambient temperature and heart rate	0.5349*	0.7701***
Relative humidity and rectal temperature	-0.4842 ^{NS}	-0.5125*
Relative humidity and respiratory rate	-0.2643 ^{NS}	-0.2928 ^{NS}
Relative humidity and heart rate	-0.2217 ^{NS}	-0.3749 ^{NS}
Temperature humidity index and rectal temperature	0.6531*	0.6070*
Temperature humidity index and respiratory rate	0.8498***	0.8026***
Temperature humidity index and heart rate	0.5067*	0.7504**

^{NS} = non-significant correlation (P > 0.05), * P < 0.05, ** P < 0.01, *** P < 0.001.

The correlations between AT and physiological parameters during the journey, especially in the control donkeys were significant ($P < 0.05$). Similarly the relationships between THI and the physiological parameters were positive and significant ($P < 0.05$) in the control group. The relationship between the thermal environmental parameters after four hours of road transportation is shown in Table 4.4. The correlations between AT and physiological parameters were positive and significant in the tested donkeys. The relationship between RH and the physiological parameters were not significant.

4.5 EFFECTS OF ADMINISTRATION OF ASCORBIC ACID, HANDLING, LOADING AND ROAD TRANSPORTATION ON HAEMATOLOGICAL PARAMETERS OF DONKEYS

Tables 4.5 and 4.6 show the effects of handling, loading and four hours of road transportation on haematological parameters of test and control donkeys during the harmattan season. The parameters in the test donkeys were not significantly different throughout the experimental period, although the values fluctuated at different times of the experiment.

The PCV values recorded during the experiment, pre-transportation and post-transportation were not significantly different in both the control and test donkeys. The Hb values obtained pre- and post-transportation were not significantly different. Total protein concentrations recorded in the test and control donkeys recorded show no significant difference between the pre-loading and post-loading values. However, there was a significant ($P < 0.05$) decrease in the values obtained immediately after transportation in both test and control donkeys compared to the after loading values.

Table 4.5: Haematological parameters of test donkeys transported by road (Mean \pm SEM, n = 8)

Parameters	Before transportation	After loading	After transportation	Day 7 post-transportation
Packed cell volume (%)	33.5 \pm 1.8	31.9 \pm 1.7	32.0 \pm 1.7	30.9 \pm 1.0
Haemoglobin (g %)	11.1 \pm 0.6 ^a	10.6 \pm 0.6 ^a	10.6 \pm 0.6 ^a	10.3 \pm 0.3 ^a
Total protein (g/dl)	9.8 \pm 0.1 ^a	9.8 \pm 0.3 ^a	7.0 \pm 0.3 ^b	8.4 \pm 0.2 ^c
White blood cell (x 10 ³ / μ L)	7.5 \pm 0.6 ^a	8.7 \pm 0.9 ^b	7.0 \pm 0.3 ^a	7.9 \pm 0.9 ^a
Neutrophils (x 10/ μ L)	50.5 \pm 4.2 ^a	58.6 \pm 4.2 ^a	40.6 \pm 2.5 ^b	52.13 \pm 5.4 ^a
Lymphocytes (x 10 ³ / μ L)	37.6 \pm 4.8	35.3 \pm 4.8	50.9 \pm 3.3	40.0 \pm 5.4
Monocytes (x 10 ³ / μ L)	5.4 \pm 1.2	2.6 \pm 0.5	2.0 \pm 0.4	3.4 \pm 0.8
Eosinophils (x 10 ³ / μ L)	5.5 \pm 1.2 ^a	4.7 \pm 0.9 ^a	4.3 \pm 0.4 ^a	4.1 \pm 0.8 ^a
Band (x 10 ³ / μ L)	1.8 \pm 0.3	1.0 \pm 0.0	1.5 \pm 0.3	1.5 \pm 0.3
Neutrophil:Lymphocyte ratio	1.6 \pm 0.3 ^a	2.1 \pm 0.6 ^a	0.80 \pm 0.1 ^b	1.9 \pm 0.7 ^a
Basophils (x 10 ³ / μ L)	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00

^{a, b, c} = Mean values with different superscript letters are significantly (P < 0.05) different.

Table 4.6: Haematological parameters of control donkeys transported by road (Mean \pm SEM, n = 5)

Parameters	Before transportation	After loading	After transportation	Day 7 post-transportation
Packed cell volume (%)	30.6 \pm 2.0	32.8 \pm 1.4	30.0 \pm 1.9	33.6 \pm 1.7
Haemoglobin (g %)	10.1 \pm 0.7	10.9 \pm 0.5	9.9 \pm 0.7	11.2 \pm 0.6
Total protein (g/dl)	8.7 \pm 0.2 ^a	8.8 \pm 0.6 ^a	6.9 \pm 0.8 ^b	8.1 \pm 0.2 ^a
White blood cell (x 10 ³ / μ L)	7.3 \pm 0.9 ^a	7.4 \pm 0.9 ^a	6.6 \pm 0.7 ^{ac}	8.4 \pm 1.0 ^{ab}
Neutrophils (x 10/ μ L)	50.4 \pm 5.2	53.5 \pm 2.8	46.0 \pm 2.6	51.2 \pm 5.0
Lymphocytes (x 10 ³ / μ L)	43.4 \pm 5.8	36.3 \pm 2.8	41.6 \pm 5.3	47.6 \pm 2.6
Monocytes (x 10 ³ / μ L)	2.6 \pm 0.2	3.0 \pm 0.7	2.0 \pm 0.4	3.5 \pm 1.0
Eosinophils (x 10 ³ / μ L)	4.3 \pm 0.9	6.4 \pm 0.9	4.0 \pm 0.5	3.8 \pm 1.1
Band (x 10 ³ / μ L)	1.0 \pm 0.0 ^a	2.0 \pm 0.0 ^b	1.5 \pm 0.3 ^c	1.0 \pm 0.0 ^a
Neutrophil:Lymphocyte ratio	1.4 \pm 0.5 ^a	1.5 \pm 0.2 ^a	1.0 \pm 0.1 ^b	1.4 \pm 0.3 ^a
Basophils (x10 ³ / μ L)	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00

^{a, b, c} = mean values with different superscript letters are significantly (P < 0.05) different.

The WBC values recorded after loading in the test donkeys increased ($P < 0.05$) from the corresponding pre-loading value, the pre- and post-loading values obtained in control donkey were not significantly different. The neutrophil values increased after loading, especially in the test group, but decreased significantly ($P < 0.05$) after transportation. The value obtained on day 7 after transportation was not significantly different from the pre-loading values. Although the lymphocyte values in control and test donkeys decreased after loading, the value rose after the journey. The values are higher in the test donkeys, compared to the control. Monocyte and eosinophil values were not significantly different throughout the experiment. The N/L ratio increased post-loading in both the test and control donkeys but the ratio was higher ($P < 0.05$) in the test group. The values obtained immediately after transportation was lower than the post-loading values in both the test and control donkeys. However, the post-loading values in test donkey was lower ($P < 0.05$) than that of the control donkey. Basophil was not recorded throughout the study period.

4.6 EFFECTS OF ADMINISTRATION OF ASCORBIC ACID, HANDLING, LOADING AND FOUR HOURS OF ROAD TRANSPORTATION ON ERYTHROCYTE OSMOTIC FRAGILITY IN THE DONKEYS

Figure 4.4 shows the values of percent haemolysis of erythrocyte of test and control donkeys pre-transportation. The highest values of percent haemolysis of $88.7 \pm 6.9 \%$ and $88.3 \pm 11.7 \%$ occurred at 0.3 % NaCl concentration in test and control, respectively. Figure 4.5 shows the values of percent haemolysis of erythrocytes of the donkeys after loading.

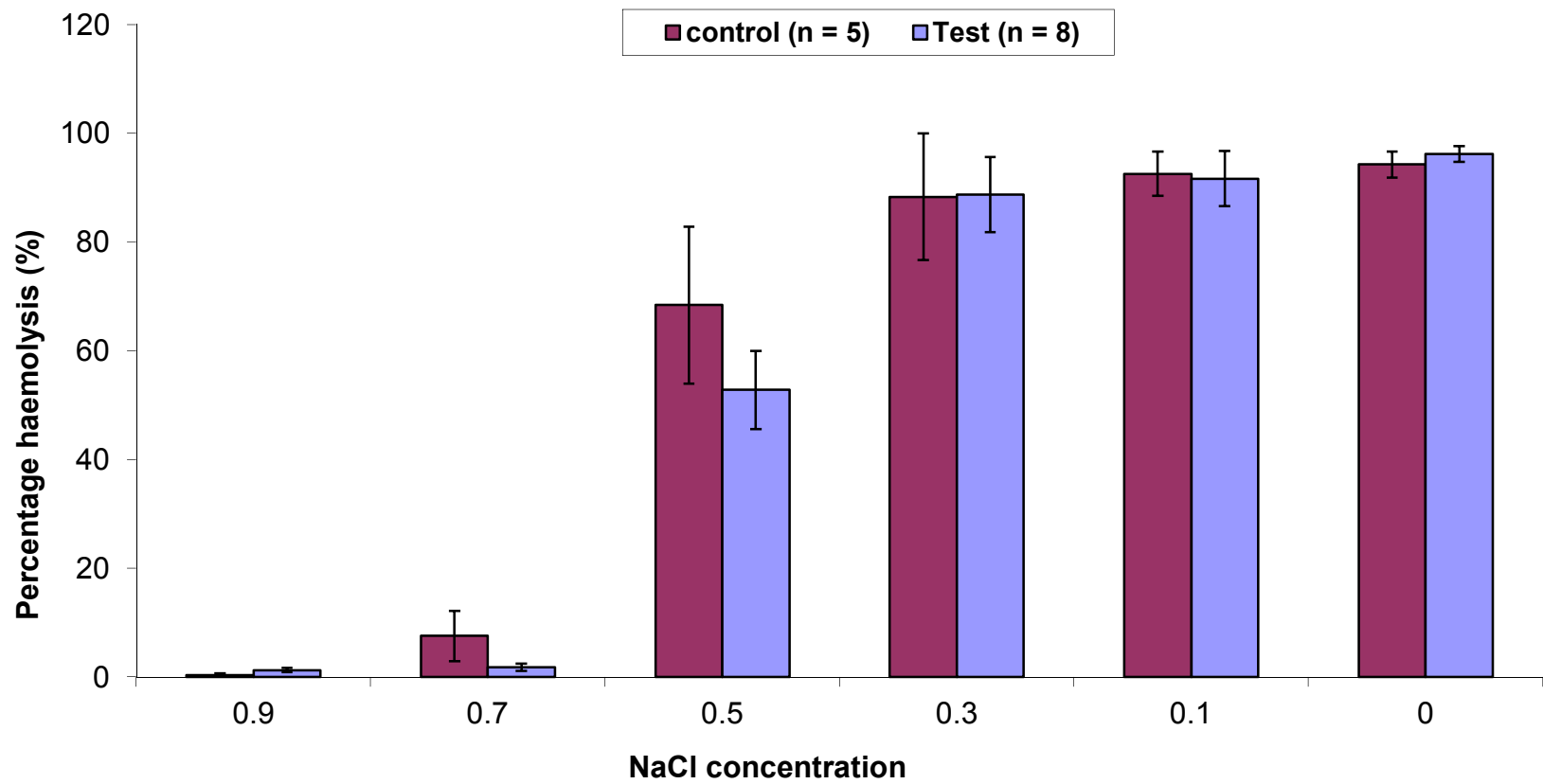


Figure 4.4: Pre-transportation erythrocyte osmotic fragility of donkeys transported by road during the harmattan season
Values are not significantly ($P > 0.05$) different.

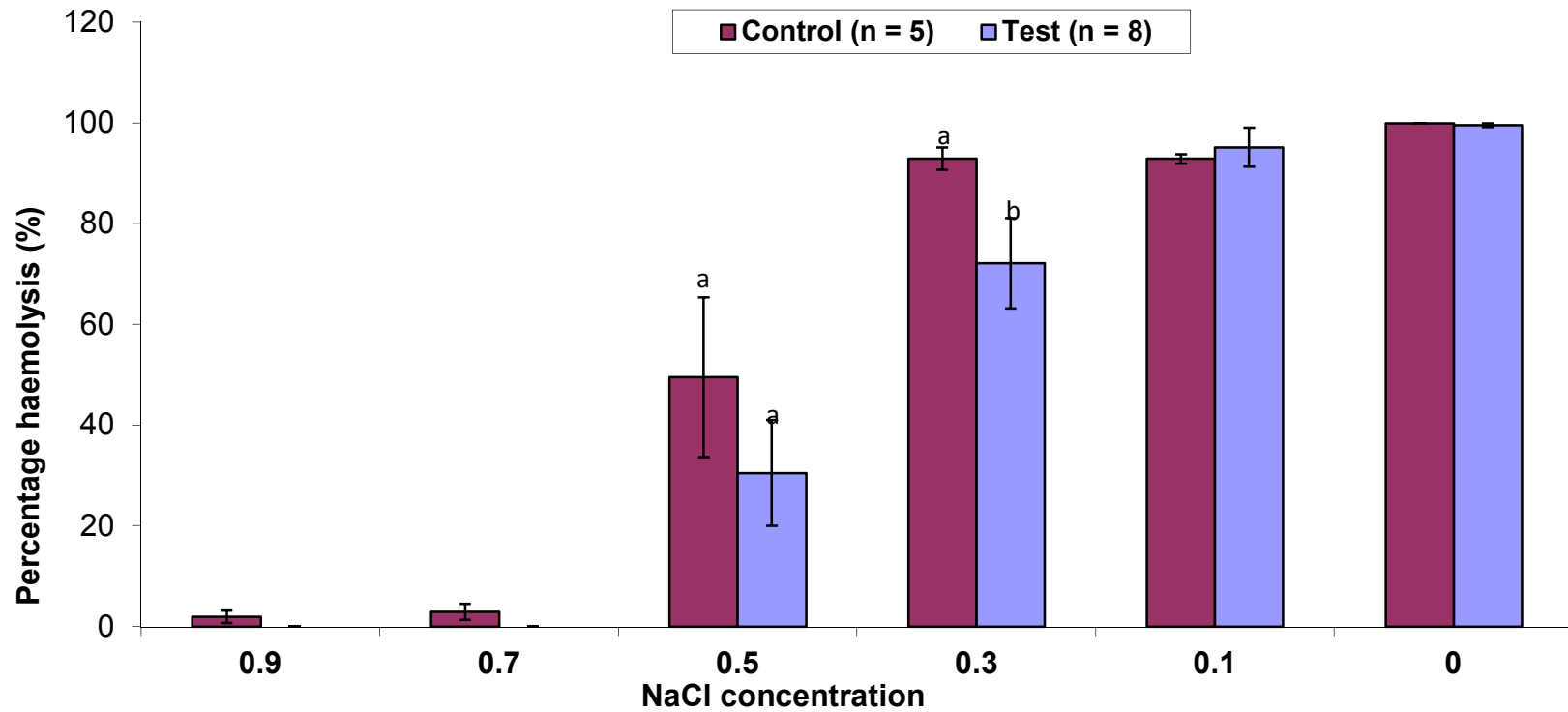


Figure 4.5: Effect of ascorbic acid on erythrocyte osmotic fragility after loading of donkeys transported by road during the harmattan season

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

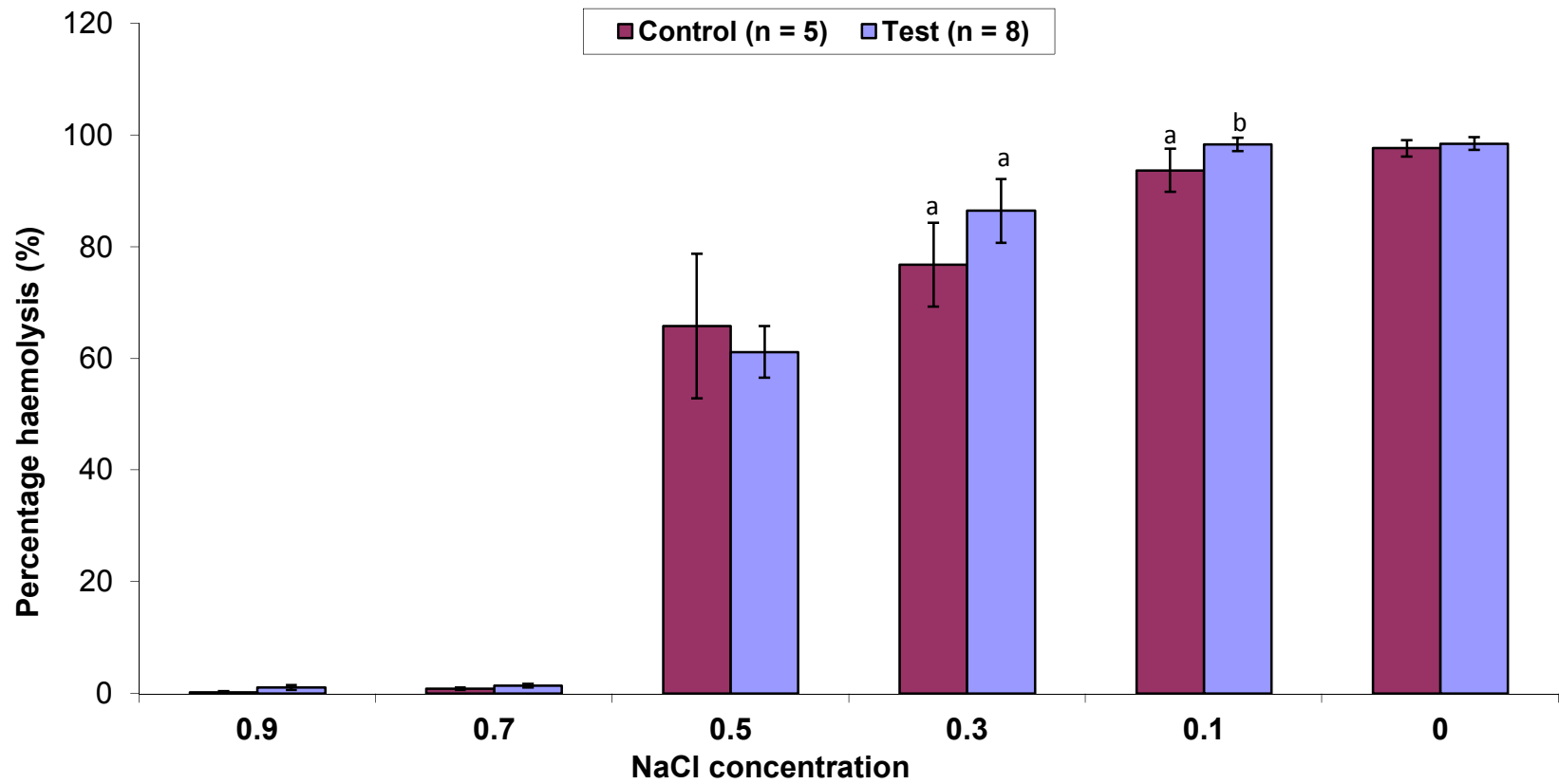


Figure 4.6: Effect of ascorbic acid on erythrocyte osmotic fragility after journey of donkeys transported by road during the harmattan season

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

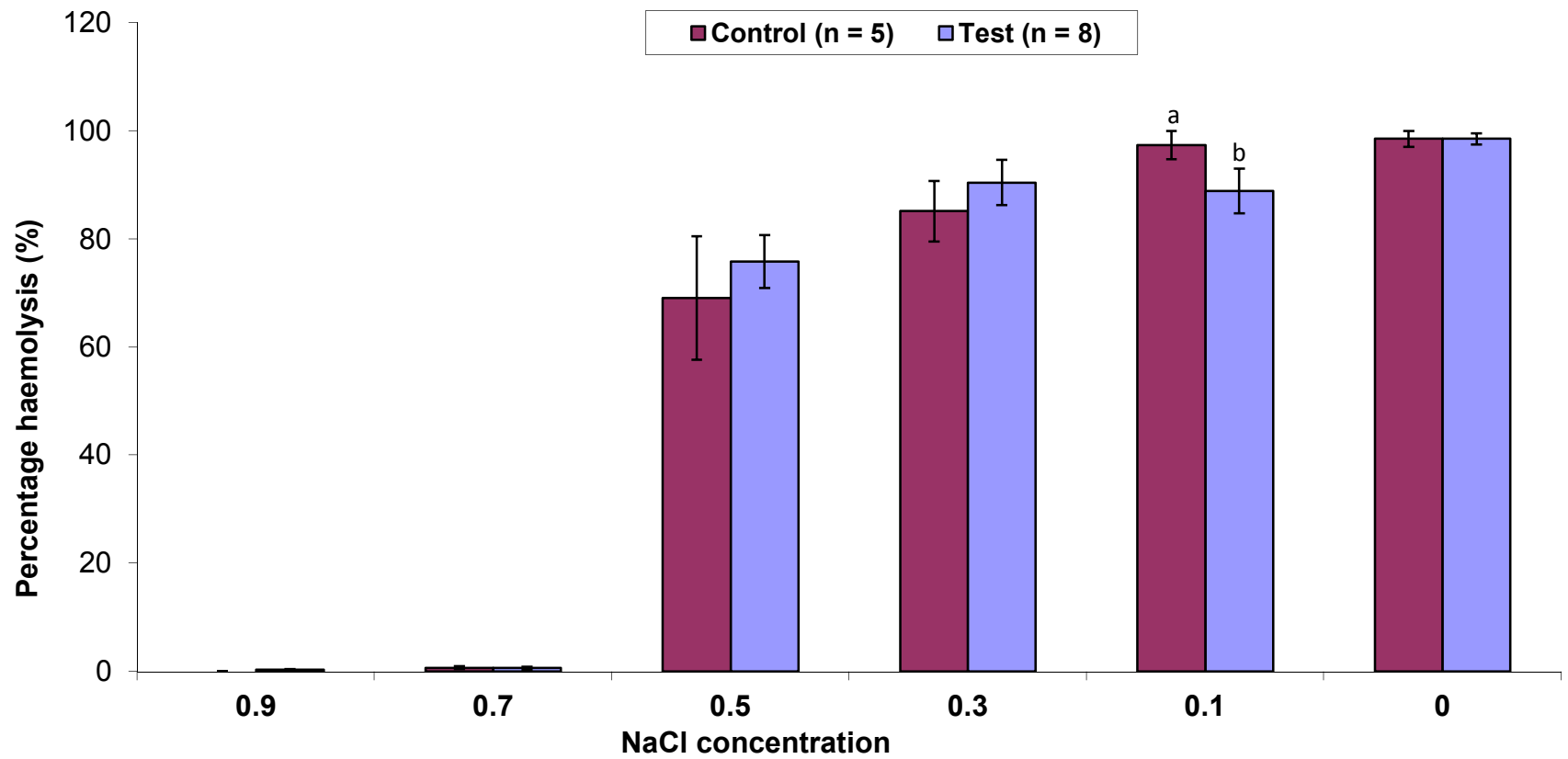


Figure 4.7: Effect of ascorbic acid on erythrocyte osmotic fragility 3 days after journey of donkeys transported by road during the harmattan season

^{a, b} = Values with different superscript letters are significantly ($P < 0.05$) different

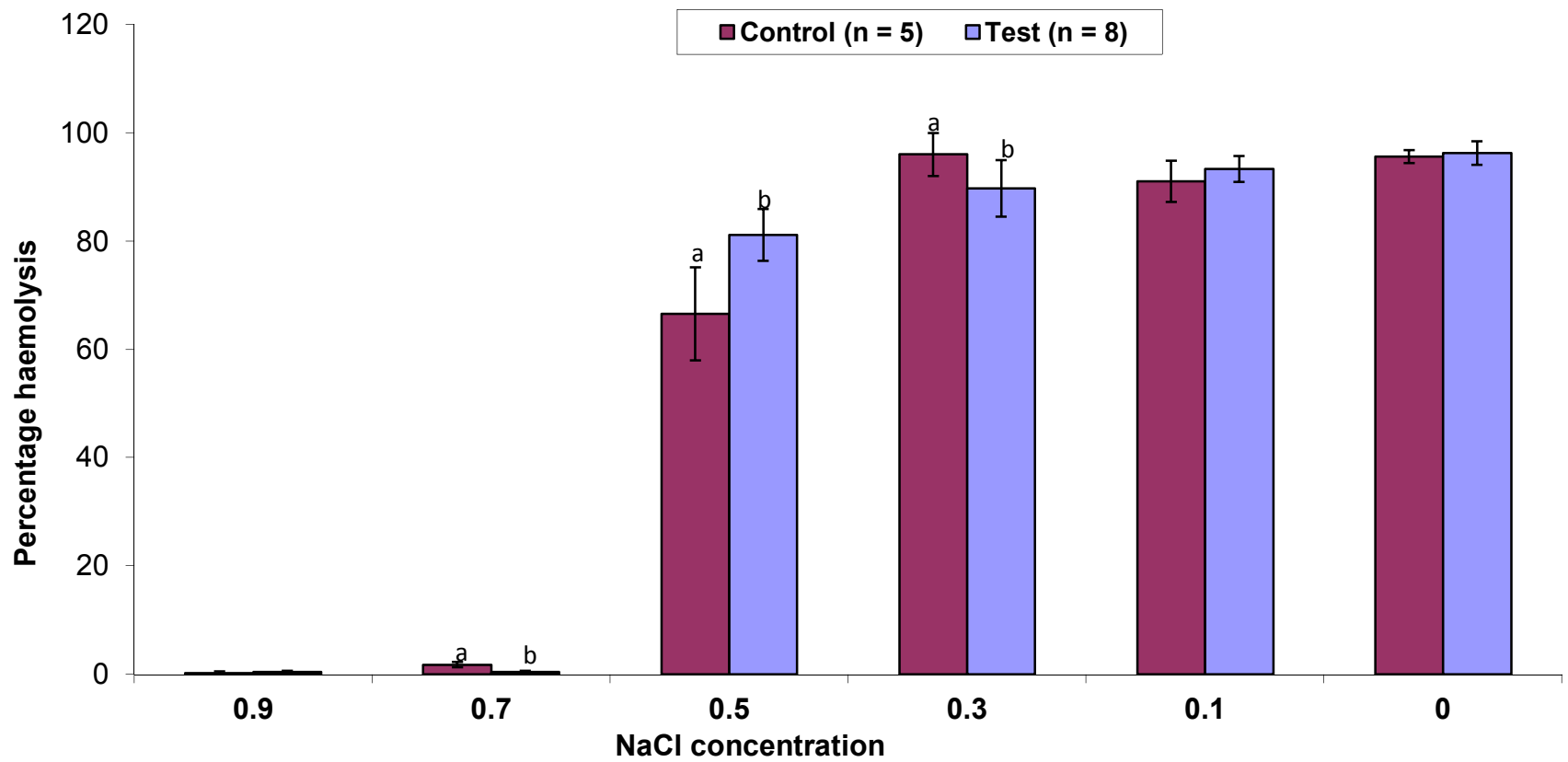


Figure 4.8: Effect of ascorbic acid on erythrocyte osmotic fragility 7 days after journey of donkeys transported by road during the harmattan season

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

The percent haemolysis in control donkeys after loading was higher ($P < 0.05$) than that of the test donkeys at 0.3 % concentration of NaCl, 92.9 ± 2.3 % and 72.2 ± 9.0 % values, respectively. The values of the percent haemolysis of the donkeys immediately after transportation are shown in Figure 4.6. The values were significantly higher ($P < 0.05$) in test than in the control group. At 0.1 % NaCl concentration, the values of the percent haemolysis in control donkeys, recorded 3 days after transportation was significantly higher ($P < 0.05$) than that of the test donkeys. Figure 4.8 shows the percent haemolysis of erythrocytes on day 7 after transportation. At 0.5 % NaCl concentration the percent haemolysis was significantly higher in the test than control donkeys.

4.7 EFFECTS OF TRANSPORTATION ON MALONDIALDEHYDE CONCENTRATION OF THE DONKEYS

Figure 4.9 shows the concentration of malondialdehyde during the experiment. The values obtained before the transportation was significantly ($P < 0.05$) higher in test donkeys than that of control. After loading, malondialdehyde concentrations in the test and control donkeys were not significantly different. Immediately after transportation malondialdehyde concentration value in the test donkeys was lower ($P < 0.05$) than that of the control.

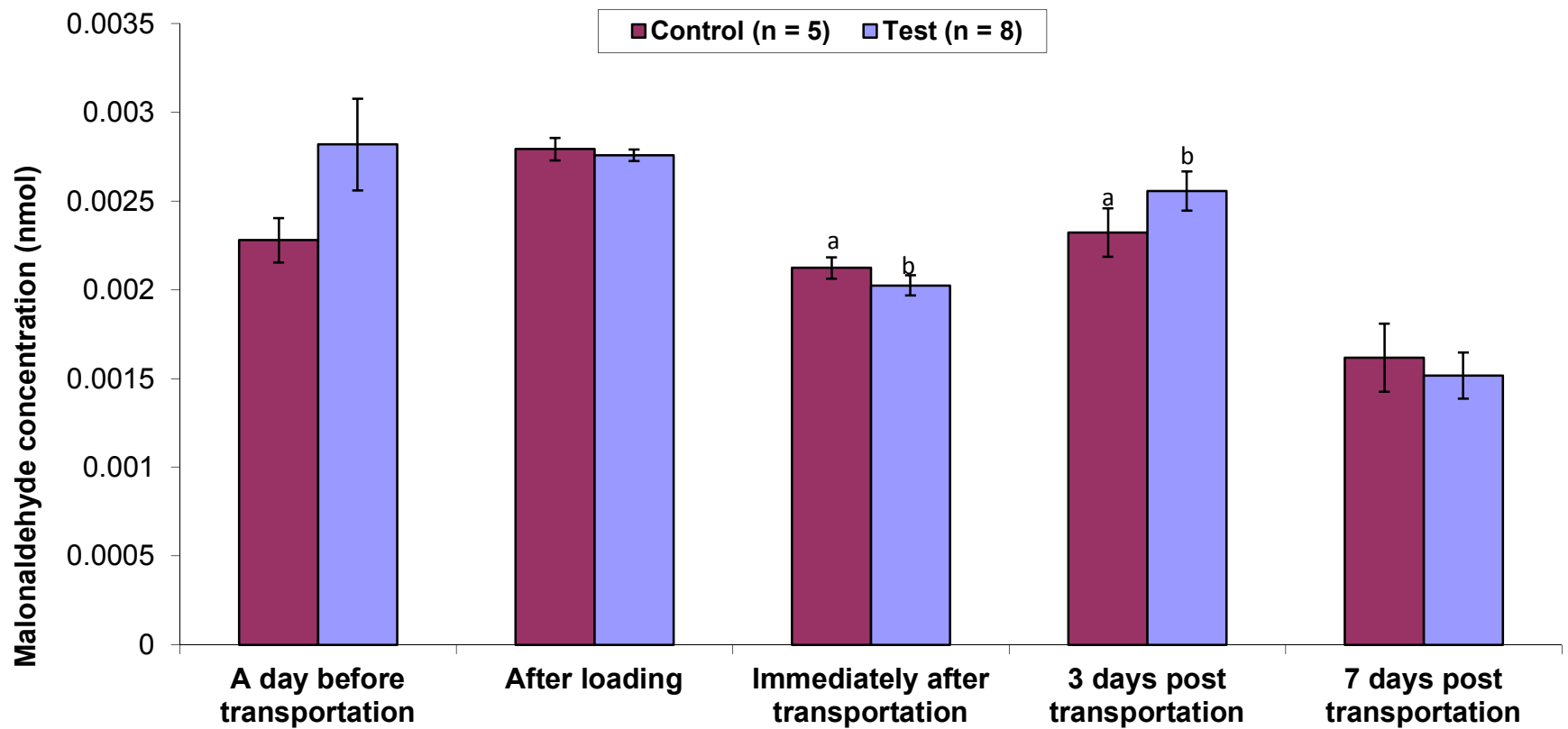


Figure 4.9: Effect of ascorbic acid on Malonaldehyde concentration of donkeys transported by road during the harmattan season

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

4.8 EFFECTS OF ROAD TRANSPORTATION ON ELECTROLYTES OF DONKEYS

Figure 4.10 shows the variations in Na concentration recorded during the experiment. After loading and immediately after transportation, the values of Na concentration recorded in the control donkeys were significantly ($P < 0.05$) higher than the corresponding values obtained in the test donkeys. On days 3 and 7 after the transportation, the values obtained in the test donkeys were significantly higher ($P < 0.05$) than those of the control donkeys.

Figure 4.11 shows the effect of the experiment on the chloride ion of the control and test donkeys. There was no significant difference in the values obtained for both control and test donkeys during and after the transportation. Effects of ascorbic acid on HCO_3^- concentration are shown in Figure 4.12. The recorded concentration of HCO_3^- in both control and test donkeys were not significantly different throughout the experiment, except on day 7 when the value obtained in the test donkeys was significantly lower ($P < 0.05$) than that of the control donkeys. Urea concentration is shown in Figure 4.13. The recorded values for urea in both the test and control donkeys were not significantly different throughout the experiment, though the values obtained were slightly higher in the control donkey. Figure 4.14 shows the values of potassium ion concentration obtained in the test and control donkeys. The value of potassium ion concentration obtained after loading in control donkeys was significantly ($P < 0.05$) lower than that recorded in the test group, after transportation the values in both the control and test donkeys were not significantly different.

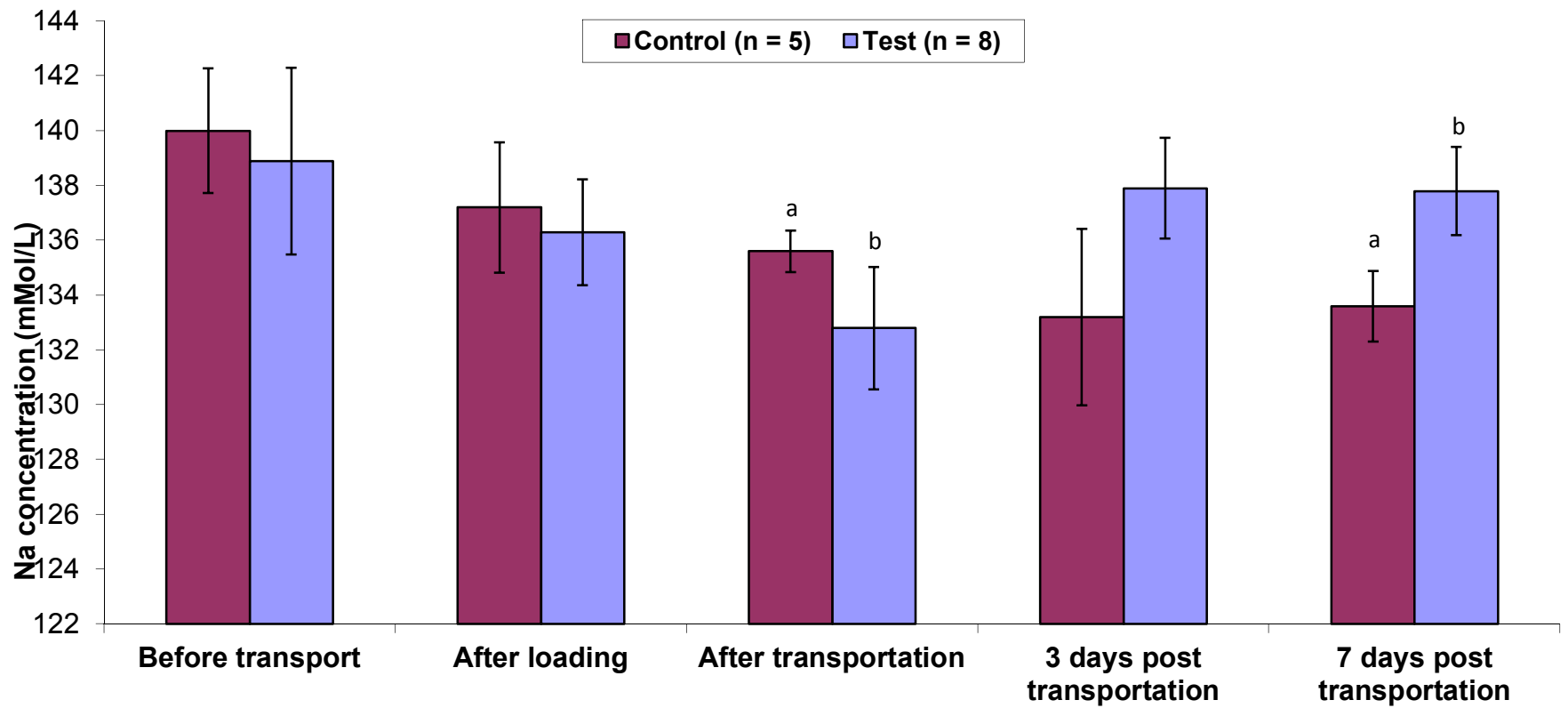


Figure 4.10: Effect of ascorbic acid on Na⁺ concentration of donkeys transported by road during the harmattan season

^{a, b} = Values with different superscript letters are significantly (P < 0.05) different.

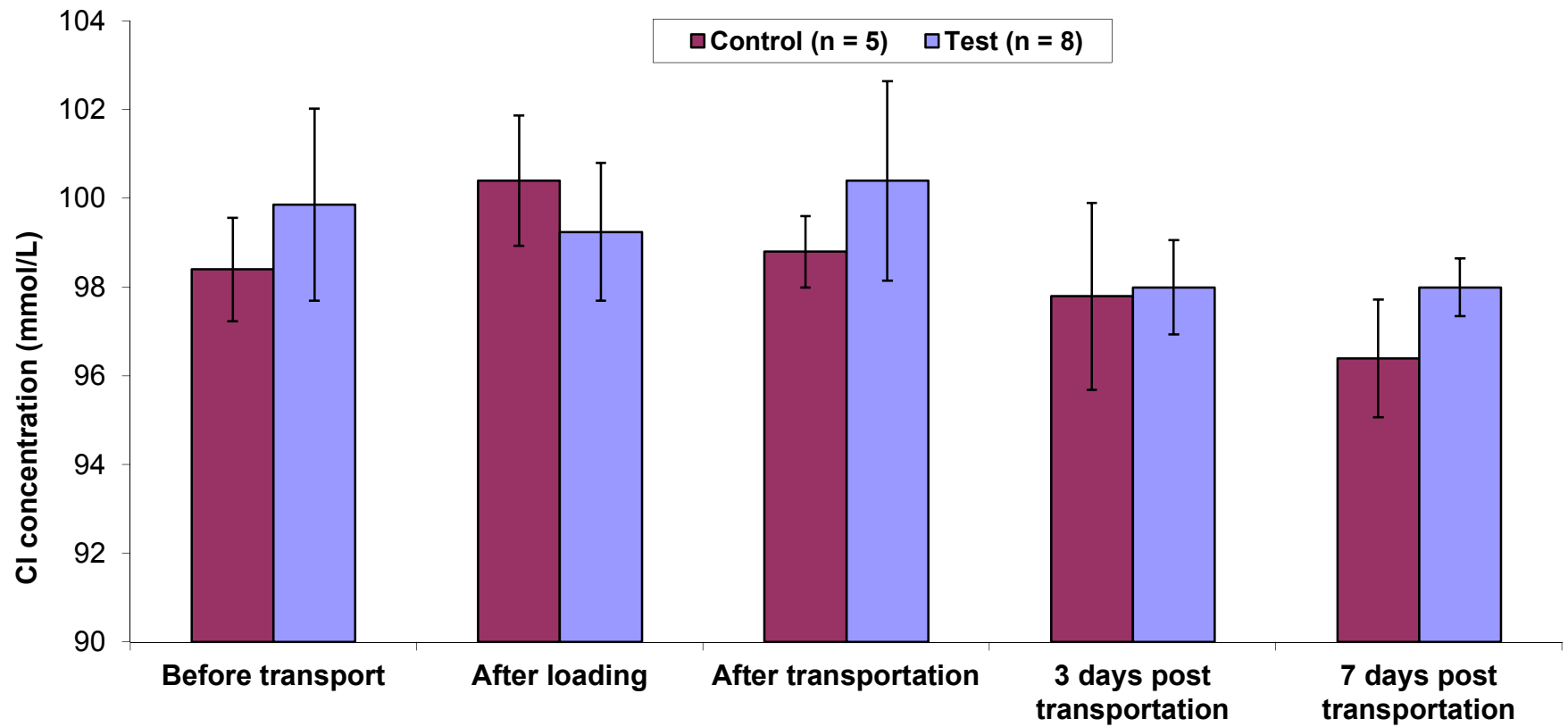


Figure 4.11: Effect of ascorbic acid on Cl concentration of donkeys transported by road during the harmattan season

Values are not significantly ($P > 0.05$) different.

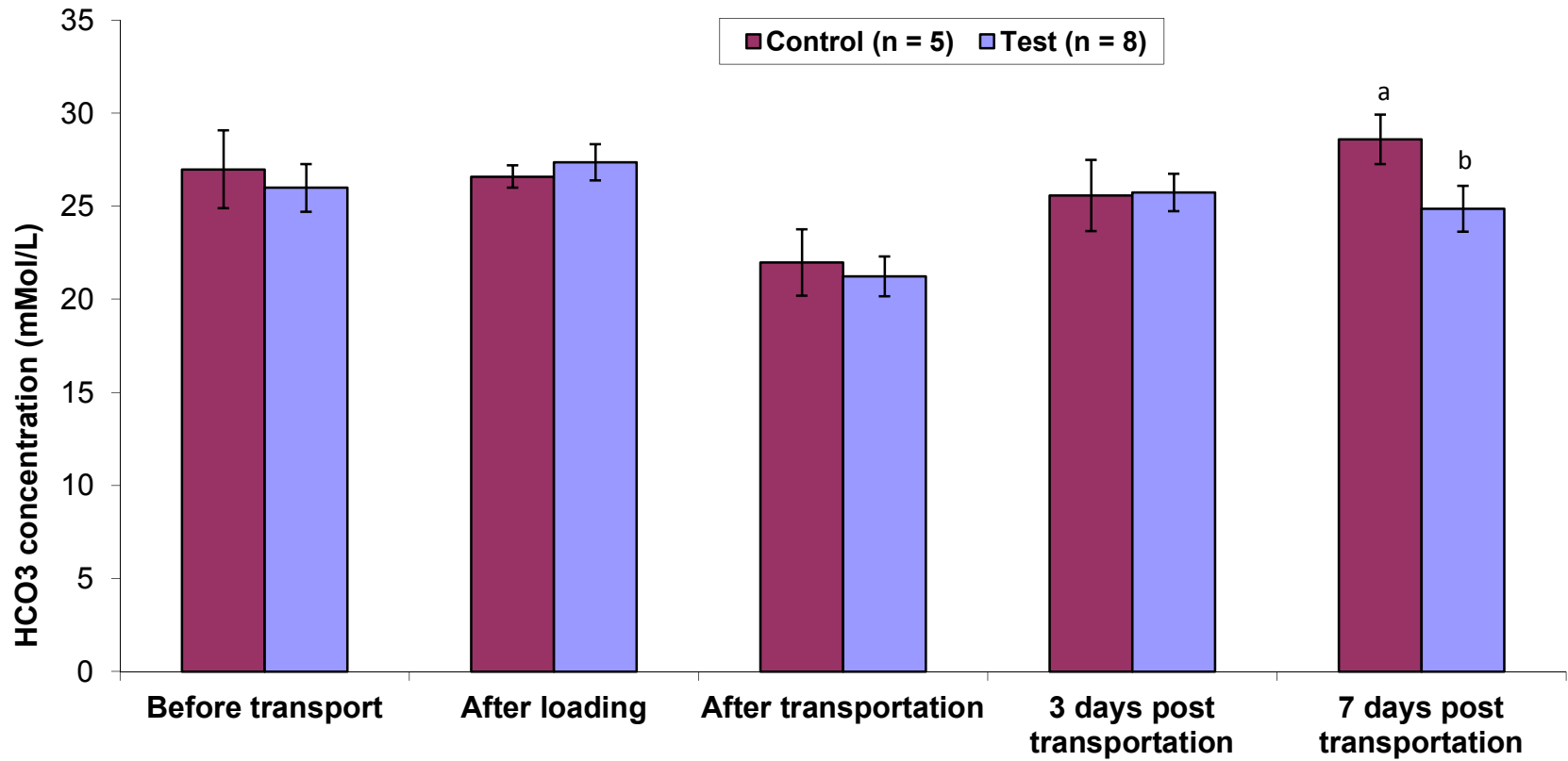


Figure 4.12: Effect of ascorbic acid on HCO_3^- concentration of donkeys transported by road during the harmattan season

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

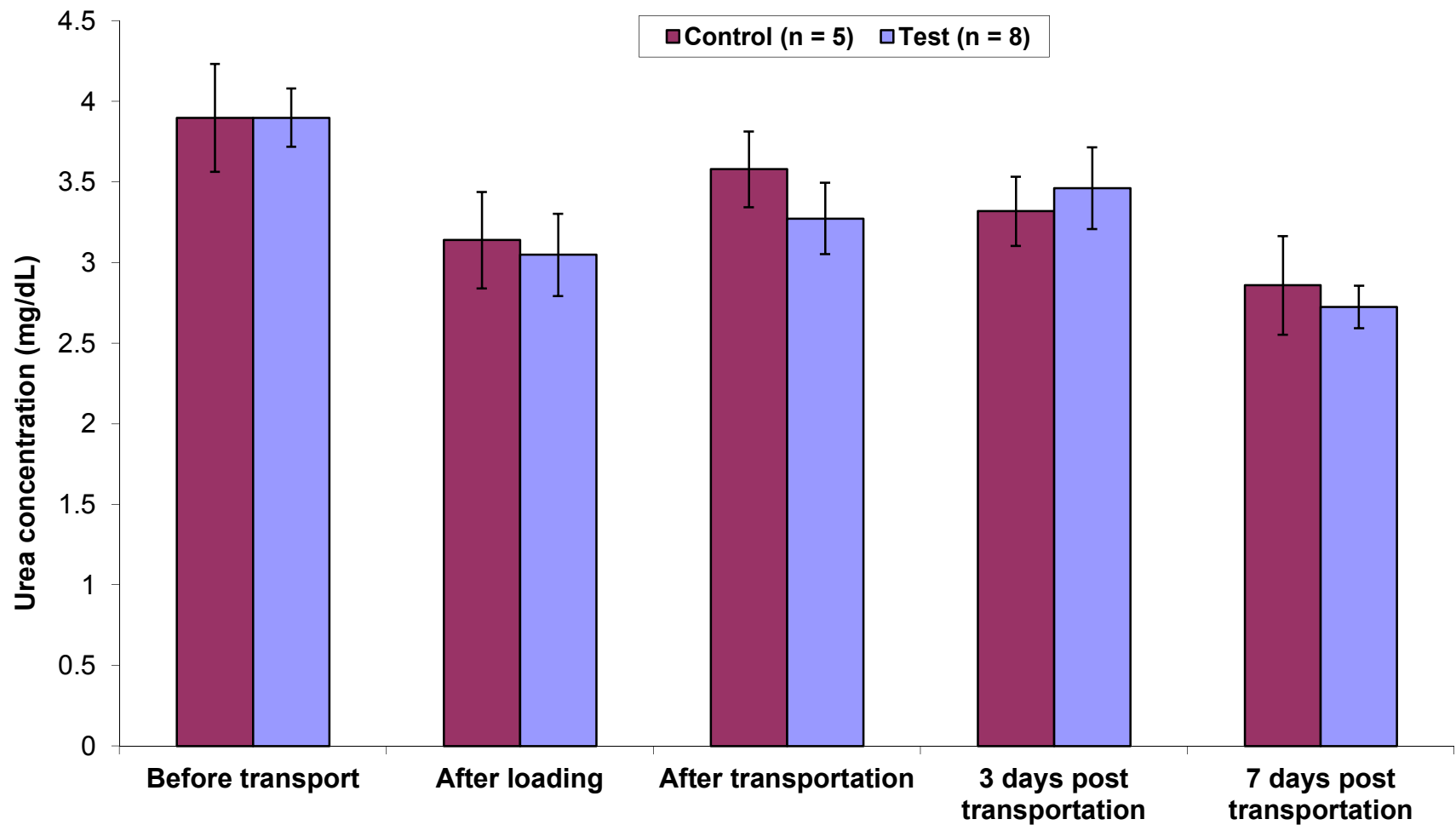


Figure 4.13: Effect of ascorbic acid on urea concentration of donkeys transported by road during the harmattan season

Values are not significantly ($P > 0.05$) different.

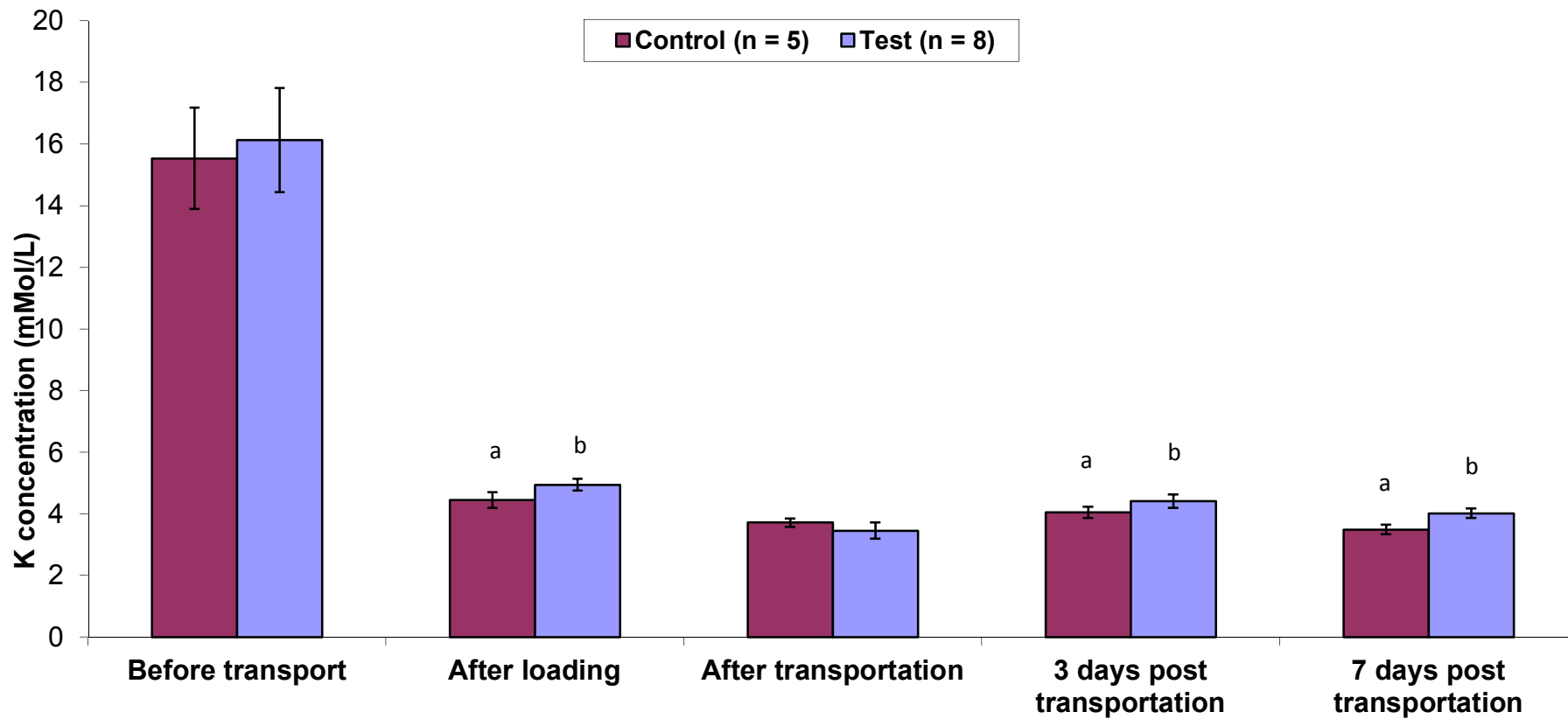


Figure 4.14: Effect of ascorbic acid on K⁺ concentration of donkeys transported by road during the harmattan season

^{a, b}=Values with different superscript letters are significantly (P < 0.05) different

CHAPTER 5

DISCUSSION

The thermal parameters obtained during the study period were characterized by low AT, RH and THI early in the mornings and evenings throughout the experimental period and high AT values during the afternoon hours, characteristic of the harmattan season in the Northern Guinea Savanna zone of Nigeria (Igono *et al.*, 1982; Ayo *et al.*, 2007). The AT may be beneficial or detrimental depending on the extent of their variations (Ayo *et al.*, 2011). The thermal environmental parameters obtained at the experimental site three days before the transportation were outside the established thermoneutral zone for the donkey; that is, AT of 23-32 °C and RH of 30-70% (Sainsbury, 1989). Bianca (1976) refers to thermoneutral zone as zone of indifference, that is, a zone within which compensatory responses by the animal are absent. During the harmattan season, wide fluctuations in AT values make the season thermally stressful and at low AT, the animal has to increase its heat production to prevent its body temperature from falling below the normal value (Bianca, 1976; Igono *et al.*, 1983). The wide range in the thermal parameters obtained from the study period imposed a great demand on the physiological responses of the animals in adjusting to the fluctuations.

The core body temperature of the animal is relatively constant and the normal diurnal range of oscillation is about 0.6-1.0°C (Piccione and Caola, 2002). The AT recorded in the early morning hours were below the established thermoneutral zone for the donkey, and the result showed that the donkeys were exposed to cold stress. Donkeys have been shown to be very adaptive animals, capable of tolerating wide fluctuations in thermal environmental parameters without any adverse effects on their physiology. Each species of animal

responds to thermal environmental challenge differently. Maintaining a constant internal temperature is the result of efficient action of thermoregulatory system (Sainsbury, 1989; Bale *et al.*, 2003; Al-Haidary, 2006). When confronted with wide fluctuations in AT, livestock compensate for variation in energy flow by altering energy intake or energy loss (David, 1980). According to Ayo *et al.* (2011), thermal environmental parameters are constantly changing, and such changes affect the physiological parameters of the animal. Thus, during the journey, high thermal environmental values were recorded, but they were within the established thermoneutral zone for the donkey. The RH value of $20.7 \pm 6.0\%$ obtained in the present study was outside of the established range of 30-70% for the donkey. RH, as one of the thermal environmental parameters, together with AT cause beneficial or detrimental effects on animals. Keim *et al.* (2002) reported that the risk of hyperthermia increases with increase in RH and AT. Dzenda *et al.* (2011) indicated a strong influence of RH on the impact of AT on values of animals RT. In this present study, the AT was not high enough to exacerbate the impact of RH on the physiological response of the donkey.

The results obtained on day 7 after the transportation were outside the thermoneutral zone for the donkey. This shows that the donkeys were further subjected to stress after the journey. The values of thermal environmental parameters throughout the study period further confirm that the harmattan season, characterized by wide fluctuations of the thermal parameters, induced both cold and heat stress in the donkeys. In this study, the afternoon hours with mean AT of 26.2 ± 1.0 °C were not extremely hot, and the values were within the normal range for the donkey.

The value of RT obtained three days before the transportation in both the test and control donkeys were within the normal range (35-39°C) for the donkey in subtropical and tropical regions. It is worth noting that the RT values recorded early in the morning were lower compared to their corresponding values recorded in the afternoon hours. The donkeys were observed to shiver in the early morning hours. The shivering observed in the donkeys was a behavioural response to low AT as internal heat was being generated in the course of shivering, and the mechanism is termed involuntary (physical) thermogenesis. This helps to maintain constant body temperature. Igono *et al.* (1982) reported shivering in goats during the harmattan season, when the AT values were below the critical temperature for the animals.

The RT, RR and HR obtained before the transportation showed that the donkeys were fit for the transportation. The post-transportation RT values obtained in the test donkeys in this study, though within the normal range of 35 – 39°C established for the donkeys in the tropics (Fielding and Krause, 1998), were higher than those of the control donkeys throughout the study period. This may be due to AA response to low AT. Gubegrirts and Linevsky (1989) reported that AA effect on the body is related to AT values. If the value is low, AA tends to enhance heat production in the body, resulting in increased RT, and if they are high, AA exerts hypothermic effects. Ayo *et al.* (1996) stated that there is indirect relationship between AT and metabolic rate in the body. During low AT, metabolic rate increases, while at high AT it decreases. The RR values increased after loading, which may be attributed to handling and loading activities. This is in agreement with the finding of Jean (1993) that RR changes rapidly due to external factors such as AT or intense activity by the animal. Forhead *et al.* (1995) also reported increase in RR 15 minutes after loading

and throughout the journey in donkeys transported by road for 4 hours. The RR values obtained in the present study were below the established range of 22-30 breaths/min for donkeys in the tropics (Fielding and Krause, 1998) and that obtained by Ayo *et al.* (2008) in donkeys during the early rainy season in the Northern Guinea Savanna zone of Nigeria. This may be attributed to the difference in thermal environmental parameter prevailing during the different seasons. The rise in RR values observed in both the test and control donkeys after loading show that the physiological responses of donkeys were in accordance with the changes in the thermal environmental parameters and loading procedures. HR values of 42 beats/min obtained after loading in the test donkeys was lower than the corresponding pre-loading value of 47.2 beats/min, while the values obtained pre- and post-loading in the controls were not different. This may be due to the effect of cortisol on HR. Forhead *et al.* (1995), recorded increase in cortisol immediately after loading. In the present study the decreased value in the test donkeys may be due to inhibitory effect of AA on cortisol thereby preventing its action in increasing the heart rate after sudden exposure to activities that can cause stress to the animals, like handling and loading procedures.

The HR values in this study at some point were higher than the established range of 38-45 beats/min for donkeys (Fielding and Krause, 1998). This may be due to the differences in seasons and times of recording. Indeed, concomitant rise in RT and RR has been reported in donkeys (Minka and Ayo, 2007b). The diurnal variation observed in RT, RR and HR values agrees with the findings of Piccone and Caola (2002), Piccione *et al.* (2007) and Dzenda *et al.* (2011) that such a fluctuation was classical of most mammals, and that it is driven by a biological clock in the hypothalamus. In the present study, the donkeys were observed to shiver early in the morning, which further confirms the low AT, characteristic

of early morning hours during the harmattan season, was below the thermoneutral zone. Thus, donkeys attempted to compensate for this by the thermogenic shivering response and increased muscular activities (Keim *et al.*, 2002). The result of the present study showed that donkeys as homeotherms have successfully adapted to the wide fluctuations in thermal environmental parameters in the zone, especially during the harmattan season. Thus, further subjection to stress during the season due to road transportation did not alter significantly the physiological parameters of the donkeys.

The relationships between the thermal environmental and physiological parameters in this study further show the importance of environmental influence on the physiological parameters, which were within normal range. There was no strong correlation between the thermal environmental and physiological parameters of both the test and control donkeys before and after the transportation but there was stronger correlation between the thermal environmental and physiological parameters of control donkeys compared to that of the test donkeys during the transportation, this is due to the ameliorative effect of AA in the experimental donkeys, reducing the stressful impact of thermal environmental. The fluctuations observed in the PCV in this study and other haematological parameters of the donkey were not significant. The parameters were within the normal values established for the donkeys in tropical and subtropical regions. A change in PCV is an indication of alteration in fluid balance that reflects dehydration or distress (Tadich *et al.*, 2005; Minka and Ayo, 2010a). The result obtained in this study indicated that the donkeys, both the test and control were not dehydrated. According to Fielding and Krause (1998), the donkey can tolerate up to 30% dehydration. It is worth noting that the PCV increased in the control donkeys immediately after loading, which is in agreement with the results obtained by

Knowles *et al.* (1999), Tadich *et al.* (2005) and Minka and Ayo (2010a). The PCV result obtained also agreed with the findings of Broom *et al.* (1996) and Minka and Ayo (2007c), who reported abrupt fall in PCV in transported animals. However in the test donkeys, there was an increase in PCV after transportation. It appears, therefore, that there are no consistencies in the direction of change in PCV values following road transportation (Minka and Ayo, 2010b).

The haemoglobin value recorded in this study further indicated that the donkeys were not dehydrated, as there was no significant difference in the values of haemoglobin obtained throughout the study. Under stress, ROS are generated, resulting in destructive effect on the erythrocyte membranes and leading to haemolysis (Altan *et al.*, 2003). The total protein value obtained in this study showed a decrease immediately after transportation, but it returned to normal on day 7. The finding disagreed with that obtained by Minka and Ayo (2010a), who reported increase in total protein after 12 hours of road transportation in goats. Forhead *et al.* (1995) reported no increase in total protein after four hours of road transportation of donkeys. The result of the present study, thus, suggested that the transported donkeys were not dehydrated, as an increase in total protein is used as an indicator of dehydration (Minka and Ayo, 2010b).

In the present study, handling and loading increased the value of neutrophils, and decreased the lymphocyte counts in the control and test donkeys, but the changes were not significant. The initial rise in neutrophils and decrease in lymphocytes may be due to the effects of cortisol released during the alarm stage of stress, which in this study occurred, apparently, during handling and loading of the donkeys. The decrease in neutrophils recorded after the

transportation disagreed with the result obtained by Minka and Ayo (2007c), who observed a steady increase in neutrophils count during transportation of goats by road during the hot-dry season. The difference in the result may be due to species and season of transportation. The result of leucocytes counts showed a significant decrease ($P < 0.05$) in N/L ratio in test donkeys after transportation. Since increase in N/L ratio is the most common index of stress from blood analysis (Stull, 1999; Minka and Ayo, 2010a), the result obtained in this study showed that AA administration reduced the adverse effects of transportation stress on the test donkeys.

The number of eosinophils recorded in this study decreased progressively after loading, but was not significant, especially in the tested animals. This finding agrees with the result of Minka and Ayo (2010b), who reported a decrease in eosinophil values after loading.

There was no significant difference in the values of monocytes and basophils obtained during handling, loading and transportation in both test and control donkeys. It has been established that stress induces haemolysis (Altan *et al.*, 2003; Asala *et al.*, 2011). In the presence of natural antioxidants erythrocytes lysis are not significant (Lee *et al.*, 2003). The innate or natural antioxidants detoxify the ROS generated by the body during stressful conditions. In the present study, administration of AA before transportation decreased the percent of haemolysis, especially at 0.3 % in the test donkeys compared to that of the control donkeys. Thus, the degree of haemolysis expressed in percentage may serve as an indicator of stress due to road transportation in donkeys.

Malondialdehyde are products of oxidative stress, used as biomarker of oxidative stress (Lee *et al.*, 2003). In this study, the values recorded after handling and loading were not

significant. The values obtained after the transportation decreased in both control and test donkeys, and the values obtained for the test donkeys were significantly ($P < 0.05$) lower than that of the control, but three days after the transportation the control donkeys values were significantly lower than that of the test donkeys. The result of the present study suggests that AA administration prior to road transportation in the test donkeys decreased lipoperoxidation, especially after transportation, but inhibitory effects of AA three days after transportation may be the reason for the higher values in the test donkeys compared to that of control donkeys. The result is in agreement with that obtained by Nazifi *et al.* (2009), who transported camels by road for 5 hours and reported a rise in malondialdehyde concentration immediately after transportation and the increase remained high for three days after the transportation. The result of the present study, however, disagreed with the finding of Burke *et al.* (2008), who recorded no increase in malondialdehyde concentration of beef calves after 2 hours of road transportation. The difference in the results may be attributed to differences in species and duration of transportation.

The increase in Na^+ in the present study showed that Na ion concentration was higher in control donkeys after loading and after the transportation, which may facilitate an increase in excitability in the control animals (Minka and Ayo, 2006). However, three days following the transportation Na^+ concentration in the control donkeys was lower than that of the test donkeys, indicating a reversed response and the inhibitory role of vitamin C, shown to possess a GABAergic effect (Karanth *et al.*, 2000; Ayo *et al.*, 2006). There was no significant difference in the value of urea obtained throughout the experimental period. This result is in agreement with the finding of Forhead *et al.* (1995), who observed no significant change in urea concentration of donkeys, transported for four hours by road.

This finding indicates that the breakdown of protein and nucleic acids in the muscles due to increase in cortisol concentration and prolonged food deprivation that usually occur in stressful transportation conditions (Buckham Sporer *et al.*, 2008; Minka and Ayo, 2010b) were not recorded in the present study.

There was significant ($P < 0.05$) decrease in the value of K^+ obtained in control donkeys, especially after loading, day 3 and 7 after transportation compared to that of the test donkeys. This result agrees with the finding of Ayo *et al.* (2009), who reported a decrease in the value of K^+ concentration in control compared with test goats after 12-h of road transportation. This was attributed to muscular damage, especially the skeletal muscles after transportation. Potassium is released from working muscles under stress or exercise due to glycogen depletion, changes in membrane permeability and increased cellular turnover (Parker *et al.*, 2003). The result of the current study suggests that administration of AA prior to 4 hours of road transportation alleviated the stressful effects of road transportation in the test donkeys.

CHAPTER 6

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY

The thermal environmental parameters during the study period were outside the thermoneutral zone established for the donkey, thus unfavourable for the donkeys. RT, RR and HR values obtained in this study followed the normal diurnal changes established for mammals. The RT values of the donkeys were within the established range for the donkey, which suggest that the donkeys were resistant. The rise in RR and HR values recorded after loading was a compensatory mechanism for energy demanding and stressful procedure. The values of haematological parameters obtained, indicate that the donkeys were not dehydrated. The decrease in N:L ratio observed was an evidence that the donkeys were able to stabilize under stress, especially in the tested donkeys due to the effect of AA.

The erythrocyte osmotic fragility in test donkeys was lower compared to that of control donkeys; AA ameliorated the destructive effects of stress on erythrocyte membrane. Malondialdehyde concentration decreased in the test donkeys compared to the control donkeys after the transportation. Na^+ concentration recorded after transportation decreased in the test donkeys compared to that of the control donkeys. There was no difference in the values of Cl^- , HCO_3^- and urea concentration obtained in both the test and control donkeys. K^+ concentration rose after the transportation in test donkeys compared to that of the control donkeys. These results indicate that the donkeys were able to cope with the changes in ion milieu, and that AA reduce the adverse effect of loading, handling and transportation in test donkeys compared to the control donkeys.

6.2 CONCLUSIONS

In conclusion, the study has shown that:

1. The harmattan season is thermally stressful to pack donkeys in the Northern Guinea Savanna zone of Nigeria.
2. Four hours of road transportation was stressful to the donkeys.
3. The pack donkeys are physiologically resistant and have adapted to the most thermally stressful conditions of the harmattan season in the zone.
4. The ameliorative effect of AA was observed especially after loading and immediately after the transportation in the test donkeys.

6.3. RECOMMENDATIONS

1. It is recommended that donkeys can be transported within the Northern States of Nigeria without any adverse effect on the donkeys, especially when administered with antioxidants before the commencement of the transportation.
2. It is recommended that further study be conducted involving transportation of donkeys for a longer duration than 4-h and for a longer distance, in order to be able to give appropriate advice to donkey transporters from the Northern Nigeria to Southern part of the country.
3. Guidelines for transporting donkeys by road should be strictly adhered to, in order to reduce the adverse effects of transportation stress in the animals.
4. Administration of antioxidants agents can alleviate the stress, induced by road transportation of donkeys from the Northern Nigeria to the Southern parts of the country. This requires further investigation.

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APPENDICES

Appendix 1: Rectal temperature of test donkeys 3 days before transportation

Donkeys	Rectal Temperature (°C)								
	Day one			Day two			Day three		
	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h
1	36.1	36.3	38.8	36.8	36.9	38.5	36.8	36.2	37.8
2	35.5	36.9	37.6	35.2	36.7	37.2	34.2	35.5	36.4
3	36.3	36.7	38.5	36.5	37.6	38.5	37.0	37.0	37.3
4	36.9	37.3	38.4	36.3	38.0	38.4	34.9	37.7	38.2
5	36.6	36.3	38.1	36.1	36.8	37.3	34.7	37.2	38.1
6	36.4	37.0	39.0	36.3	37.2	38.0	35.9	36.8	38.3
7	36.7	36.1	38.5	36.6	36.8	39.0	36.2	34.5	38.2
8	36.6	37.3	38.4	35.8	37.5	38.1	34.9	37.7	38.2

Appendix 2: Rectal temperature of control donkeys 3 days before transportation

Rectal Temperature (°C)									
Donkeys	Day one			Day two			Day three		
	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h
1	35.3	37.4	38.2	35.2	36.0	38.2	34.3	35.6	37.6
2	34.3	37.1	38.7	34.9	37.0	38.7	34.5	37.2	38.0
3	36.3	36.5	38.1	36.3	37.7	37.9	36.3	37.7	38.6
4	35.4	36.8	39.2	36.6	37.1	38.8	36.4	37.7	38.6
5	36.5	37.2	38.4	36.4	37.2	38.3	36.0	37.3	38.0

Appendix 3: Rectal temperature of test donkeys during transportation (°C)

Donkeys	After loading	1 Hour into the journey	2 Hours into the journey	Immediately after the journey
1	36.4	36.8	37.8	38.0
2	35.6	36.1	36.9	37.1
3	37.4	36.9	37.4	38.3
4	35.5	36.7	36.9	38.2
5	36.6	35.7	36.8	37.6
6	36.5	36.5	37.3	38.0
7	34.4	37.4	37.0	38.4
8	36.3	36.7	36.9	36.6

Appendix 4: Rectal temperature of control donkeys during transportation (°C)

Donkeys	After loading	1 Hour into the journey	2 Hours into the journey	Immediately after the journey
1	35.2	35.0	35.8	37.7
2	33.9	35.6	36.8	37.8
3	36.9	36.0	36.4	37.5
4	36.5	35.0	36.3	38.0
5	35.9	36.1	36.2	37.3

Appendix 5: Rectal temperature of test donkeys 3 days and day 7 after transportation

Donkeys	Rectal Temperature (°C)											
	Day one			Day two			Day three			Day seven		
	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h
1	35.6	37.7	37.3	32.8	36.1	35.6	34.4	35.3	37.3	34.7	36.6	37.5
2	32.5	37.4	36.0	34.4	35.1	34.6	33.2	35.2	37.3	34.3	36.6	37.0
3	36.6	36.0	37.9	35.0	36.3	35.8	35.7	36.4	36.6	35.4	37.4	37.7
4	37.1	36.0	37.6	36.2	34.9	34.6	34.3	36.0	37.0	35.6	37.0	36.9
5	36.3	34.1	36.7	34.1	34.1	35.4	33.5	35.9	36.5	36.0	36.1	36.5
6	36.2	36.1	37.5	33.9	36.1	36.6	35.1	35.5	37.6	36.4	35.6	37.5
7	34.6	35.3	37.2	35.0	33.8	36.8	34.7	36.3	37.0	35.8	34.9	36.5
8	35.6	37.8	37.2	34.8	35.5	35.3	34.0	35.3	36.8	35.4	37.0	36.6

Appendix 6: Rectal temperature of control donkeys 3 days and day 7 after transportation

Donkeys	Rectal Temperature (°C)											
	Day one			Day two			Day three			Day seven		
	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h
1	34.2	36.0	37.1	33.5	32.4	33.9	33.0	32.7	35.6	33.5	34.6	36.5
2	32.8	36.1	36.7	33.1	34.7	36.3	32.5	35.4	35.8	34.6	34.9	37.9
3	33.1	35.9	36.6	34.8	35.8	35.8	33.5	35.6	37.0	36.6	36.3	37.1
4	36.4	35.9	37.5	35.3	36.4	35.8	34.4	36.9	36.9	36.1	36.0	37.6
5	35.3	37.1	37.2	35.8	37.4	37.4	36.2	35.9	37.3	35.7	36.4	38.0

Appendix 7: Respiratory rate of test donkeys 3 days before transportation

Respiratory rate (Breaths/min)									
Donkeys	Day one			Day two			Day three		
	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h
1	16	15	16	9	17	21	11	17	11
2	12	25	25	10	39	20	12	23	21
3	9	30	18	8	17	18	12	15	11
4	8	31	14	10	32	21	10	20	14
5	16	22	27	8	17	10	8	14	12
6	12	23	19	12	26	16	9	19	12
7	12	15	25	8	19	18	7	16	19
8	10	35	13	7	27	23	9	16	15

Appendix 8: Rectal temperature of control donkeys 3 days before transportation

Respiratory Rate (Breaths/min)									
Donkeys	Day one			Day two			Day three		
	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h
1	16	27	17	11	21	16	10	22	15
2	8	20	25	7	18	20	9	12	17
3	8	23	15	10	20	21	11	14	19
4	11	20	25	8	24	13	12	21	16
5	10	15	19	11	21	20	9	21	17

Appendix 9: Respiratory rate of test donkeys during transportation (Breaths/min)

Donkeys	After loading	1 Hour into the journey	2 Hours into the journey	Immediately after the journey
1	25	11	14	16
2	19	12	12	15
3	24	13	19	16
4	12	12	15	22
5	13	10	12	14
6	19	15	24	17
7	16	10	13	24
8	12	12	18	16

Appendix 10: Respiratory rate of control donkeys during transportation (Breaths/min)

Donkeys	After loading	1 Hour into the journey	2 Hours into the journey	Immediately after the journey
1	17	16	16	15
2	19	15	20	12
3	14	20	16	18
4	18	10	16	12
5	18	9	14	18

Appendix 11: Respiratory rate of test donkeys 3 days and day 7 after transportation

Donkeys	Respiratory Rate (Breaths/min)											
	Day one			Day two			Day three			Day seven		
	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h
1	10	21	20	8	14	10	7	10	9	11	21	13
2	9	58	14	10	16	19	8	10	11	9	20	20
3	8	13	13	7	10	10	9	10	9	7	16	12
4	10	14	12	10	14	13	10	12	9	8	27	15
5	7	11	11	9	10	8	7	8	8	10	17	9
6	10	13	12	12	11	14	10	11	11	7	10	14
7	9	9	13	8	14	10	7	9	9	8	23	12
8	9	25	12	6	14	7	7	13	10	10	13	11

Appendix 12: Respiratory rate of control donkeys 3 days and day 7 after transportation

Donkeys	Respiratory Rate (Breath/min)											
	Day one			Day two			Day three			Day seven		
	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h
1	7	12	16	7	13	9	6	9	9	9	11	13
2	8	12	13	9	11	20	7	10	8	8	12	10
3	8	14	12	8	12	11	9	12	9	9	17	10
4	8	11	12	9	12	10	8	10	10	8	10	10
5	10	14	13	8	13	10	8	11	9	7	12	12

Appendix 13: Heart rate of test donkeys 3 days before transportation

Heart Rate (Beats/min)									
Donkeys	Day one			Day two			Day three		
	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h
1	52	62	64	48	56	60	53	52	55
2	48	50	55	39	47	42	40	41	45
3	54	70	55	42	47	49	45	50	41
4	52	56	56	40	48	44	44	50	47
5	34	56	60	40	49	50	41	49	49
6	54	62	65	42	54	53	41	52	47
7	44	44	50	36	39	43	31	44	43
8	42	46	46	38	53	46	37	47	49

Appendix 14: Heart rate of control donkeys 3 days before transportation

Heart Rate (Beats/min)									
Donkeys	Day one			Day two			Day three		
	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h
1	40	42	46	31	41	43	35	39	55
2	42	54	64	39	49	56	39	51	48
3	54	56	54	44	49	55	48	55	56
4	44	58	71	45	47	48	51	68	64
5	44	50	52	40	47	48	41	50	50

Appendix 15: Heart rate of test donkeys during transportation (Beats/min)

Donkeys	After loading	1 Hour into the journey	2 Hours into the journey	Immediately after the journey
1	51	47	52	51
2	38	30	42	42
3	55	63	49	51
4	35	37	46	49
5	45	42	46	41
6	46	47	50	57
7	30	32	35	56
8	36	38	39	46

Appendix 16: Heart rate of control donkeys during transportation (Beats/min)

Donkeys	After loading	1 Hour into the journey	2 Hours into the journey	Immediately after the journey
1	37	30	34	50
2	36	40	44	40
3	59	54	48	46
4	72	50	53	47
5	40	36	42	43

Appendix 17: Heart rate of test donkeys 3 days and day 7 after transportation

Donkeys	Heart Rate (Beats/min)											
	Day one			Day two			Day three			Day seven		
	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h
1	43	58	52	44	55	42	43	59	54	34	60	47
2	38	54	42	39	45	43	40	41	44	36	45	37
3	43	50	53	40	48	50	42	63	49	44	51	52
4	39	41	48	35	34	41	40	47	46	34	42	39
5	40	45	48	38	41	37	40	50	46	42	42	42
6	41	48	54	41	51	50	42	53	50	42	43	37
7	32	37	38	31	37	38	33	46	40	33	40	36
8	36	42	42	37	39	40	34	43	42	30	42	38

Appendix 18: Heart rate of control donkeys 3 days and day 7 after transportation

Heart Rate (Beats/min)												
Donkeys	Day one			Day two			Day three			Day seven		
	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h	06:00 h	13:00 h	18:00 h
1	30	36	40	34	34	37	33	39	40	28	34	37
2	42	38	53	43	42	49	44	67	60	34	46	50
3	41	56	54	44	52	50	53	57	51	39	40	49
4	41	47	51	39	43	46	45	50	47	43	46	42
5	44	51	52	38	47	48	40	52	48	38	40	48

Appendix 19: Haematological parameters of test donkeys a day before transportation

Donkey	PCV	Hb	WBC	Total protein	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Band
1	39	13.0	6.4	10.0	46	47	5	1	-	1
2	31	10.3	9.5	9.8	60	30	3	5	-	2
3	39	13.0	8.0	9.8	57	26	7	9	-	1
4	38	12.6	8.4	10.0	56	22	11	11	-	-
5	29	9.6	6.2	9.9	41	48	6	5	-	-
6	26	8.6	5.8	9.6	35	50	8	7	-	-
7	36	12.0	5.9	10.0	40	55	1	2	-	2
8	30	10.0	10.0	9.6	69	23	2	4	-	2

Appendix 20: Haematological parameters of control donkeys a day before transportation

Donkey	PCV	Hb	WBC	Total protein	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Band
1	38	12.6	6.9	8.8	51	41	2	6	-	-
2	29	9.6	11.0	9.0	70	22	3	5	-	-
3	29	9.6	6.5	7.9	47	47	3	2	-	1
4	26	8.6	6.0	9.0	43	54	3	-	-	-
5	31	10.3	6.3	9.0	41	53	2	4	-	-

Appendix 21: Haematological parameters of test donkeys after loading

Donkey	PCV	Hb	WBC	Total protein	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Band
1	29	9.6	6.2	9.0	47	53	-	-	-	
2	38	12.6	7.2	9.4	56	38	1	5	-	
3	27	9.0	6.5	9.0	49	43	3	4	-	1
4	29	9.6	11.0	11.0	66	27	4	3	-	-
5	30	10.0	12.2	10.0	78	15	3	3	-	1
6	38	12.6	7.0	10.2	51	43	2	4	-	-
7	-	-	-	-	-	-	-	-	-	-
8	32	10.6	10.5	10.0	63	28	4	9	-	

Appendix 22: Haematological parameters of control donkeys after loading

Donkey	PCV	Hb	WBC	Total protein	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Band
1	31	10.3	6.7	9.0	48	38	4	8	-	2
2	30	10.0	10.0	7.0	60	28	4	6	-	2
3	-	-	-	-	-	-	-	-	-	-
4	36	12.0	6.4	9.0	50	40	3	5	-	2
5	34	11.3	6.5	10.1	56	39	1	4	-	-

Appendix 23: Haematological parameters of test donkeys after transportation

Donkey	PCV	Hb	WBC	Total protein	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Band
1	25	8.3	6.7	6.0	40	57	-	3	-	-
2	38	12.6	7.0	6.2	34	60	1	4	-	1
3	-	-	-	-	-	-	-	-	-	-
4	31	10.3	6.2	8.0	35	40	2	6	-	2
5	31	10.3	6.9	8.0	44	49	2	4	-	1
6	30	10.0	7.2	7.5	34	60	-	6	-	-
7	37	12.3	9.2	7.5	50	40	3	5	-	2
8	32	10.6	5.7	6.0	47	50	-	3	-	-

Appendix 24: Haematological parameters of control donkeys after loading

Donkey	PCV	Hb	WBC	Total protein	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Band
1	34	11.3	6.0	6.0	45	50	-	5	-	-
2	35	11.6	9.5	6.0	55	38	1	5	-	1
3	26	8.3	5.3	7.2	42	50	3	3	-	2
4	28	9.3	6.1	10.0	40	53	2	4	-	1
5	27	9.0	6.0	5.5	48	47	2	3	-	-

Appendix 25: Haematological parameters of test donkeys day 7 after transportation

Donkey	PCV	Hb	WBC	Total protein	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Band
1	33	11.0	5.6	7.8	42	49	7	2	-	-
2	29	9.7	6.9	8.0	51	43	-	6	-	-
3	33	11.0	6.8	9.2	51	42	1	6	-	-
4	32	10.7	6.0	8.8	47	43	2	6	-	2
5	25	8.3	12.6	8.6	82	12	4	1	-	1
6	31	10.3	5.5	8.4	29	65	5	1	-	-
7	33	11.0	10.0	8.2	57	33	4	5	-	1
8	31	10.3	9.8	8.2	58	33	1	6	-	2

Appendix 26: Haematological parameters of control donkeys day 7 after transportation

Donkey	PCV	Hb	WBC	Total Protein	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Band
1	30	10.0	5.9	7.6	38	53	5	4	-	-
2	33	11.0	10.1	8.2	59	33	3	5	-	-
3	36	12.0	9.8	8.0	55	32	5	7	-	1
4	39	13.0	5.7	8.2	41	56	1	1	-	1
5	30	10.0	10.3	8.6	63	34	-	2	-	1

Appendix 27: Erythrocyte osmotic fragility of test donkeys a day before transportation

Donkeys	0.9	0.7	0.5	0.3	0.1	0.0
1	1.56	1.56	42.2	93.8	100	100
2	2.00	3.00	69.0	100	100	100
3	2.27	4.55	93.2	100	95.5	90.9
4	0.00	0.00	44.6	100	92.9	92.9
5	0.00	4.65	58.1	100	95.4	93.0
6	2.97	0.00	50.0	100	93.1	93.1
7	0.67	0.00	34.7	50.0	56.7	100
8	1.10	1.10	30.9	66.0	100	100

Appendix 28: Erythrocyte osmotic fragility of control donkeys a day before transportation

Donkeys	0.9	0.7	0.5	0.3	0.1	0.0
1	0.00	0.00	71.0	100	77.1	85.7
2	0.00	0.00	33.0	41.7	100	100
3	0.00	1.90	100	100	96.3	96.3
4	0.00	1.90	100	100	96.3	96.3
5	1.70	0.00	38.3	100	93.3	93.4

Appendix 29: Erythrocyte osmotic fragility of test donkeys immediately after loading

Donkeys	0.9	0.7	0.5	0.3	0.1	0.0
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	0.00	0.00	30.4	65.2	100	100
4	0.00	0.00	40.8	81.6	100	98.0
5	0.00	0.00	2.30	95.5	100	100
6	-	-	-	-	-	-
7	0.00	0.00	63.3	76.7	80.0	100
8	0.00	0.00	16.0	42.0	96.0	100

Appendix 30: Erythrocyte osmotic fragility of control donkeys immediately after loading

Donkeys	0.9	0.7	0.5	0.3	0.1	0.0
1	0.00	0.00	8.5	85.1	93.6	100
2	3.90	3.90	30.8	94.2	94.2	100
3	5.90	8.80	79.4	91.2	94.1	100
4	0.00	2.20	93.3	97.8	93.3	100
5	0.00	0.00	35.7	96.4	89.0	100

Appendix 31: Erythrocyte osmotic fragility of test donkeys immediately after transportation

Donkeys	0.9	0.7	0.5	0.3	0.1	0.0
1	0.00	1.60	66.1	80.7	96.8	100
2	1.10	1.10	54.4	64.4	100	97.8
3	2.30	2.30	58.1	100	93.0	93.0
4	-	-	-	-	-	-
5	0.00	0.00	75.0	100	100	100
6	-	-	-	-	-	-
7	2.20	2.20	69.6	93.5	100	100
8	1.30	1.30	43.8	80.0	100	100

Appendix 32: Erythrocyte osmotic fragility of control donkeys immediately after transportation

Donkeys	0.9	0.7	0.5	0.3	0.1	0.0
1	1.30	1.30	25.0	56.3	100	93.8
2	0.00	1.10	54.7	79.0	79.0	100
3	0.00	0.00	85.3	82.7	100	100
4	0.00	1.10	100	100	94.4	94.4
5	0.00	1.00	64.0	66.0	95.0	100

Appendix 33: Erythrocyte osmotic fragility of test donkeys day 3 after transportation

Donkeys	0.9	0.7	0.5	0.3	0.1	0.0
1	1.30	1.30	90.7	100	100	93.3
2	0.00	0.00	72.6	73.7	100	94.7
3	0.00	0.00	87.5	77.5	80.0	100
4	0.00	0.00	75.8	100	100	100
5	0.00	1.20	55.3	100	68.2	100
6	0.00	0.00	80.4	100	87.5	100
7	1.20	1.20	88.2	94.1	94.1	100
8	0.00	1.60	56.3	78.1	81.3	100

Appendix 34: Erythrocyte osmotic fragility of control day 3 after transportation

Donkeys	0.9	0.7	0.5	0.3	0.1	0.0
1	0.00	0.00	29.5	84.2	89.5	100
2	0.00	0.00	100	100	-	-
3	0.00	1.20	68.2	68.2	100	94.1
4	0.00	1.10	79.0	79.0	100	100
5	0.00	1.40	68.6	94.3	100	100

Appendix 35: Erythrocyte osmotic fragility of test donkeys day 7 after transportation

Donkeys	0.9	0.7	0.5	0.3	0.1	0.0
1	2.00	2.00	92.0	98.0	100	100
2	0.00	0.00	62.5	100	87.5	100
3	1.40	1.40	80.0	100	97.1	97.1
4	0.00	0.00	96.4	100	100	100
5	0.00	0.00	78.0	74.0	90.0	100
6	0.00	0.00	100	83.3	83.3	100
7	0.00	0.00	70.3	62.5	100	84.4
8	0.00	0.00	70.0	100	88.6	88.6

Appendix 36: Erythrocyte osmotic fragility of control day 7 after transportation

Donkeys	0.9	0.7	0.5	0.3	0.1	0.0
1	0.00	2.70	88.0	100	93.3	93.3
2	0.00	0.00	72.4	100	100	96.6
3	0.00	1.40	35.7	80.0	91.4	100
4	1.40	2.90	71.4	100	77.1	94.3
5	0.00	2.10	65.3	100	93.9	93.9

Appendix 37: Malondialdehyde concentration of test donkeys transported by road during the harmattan season

Donkeys	Test donkeys				
	A day Before	After loading	After transportation	Day 3 after transportation	Day 7 after transportation
1	2.64×10^{-3}	2.87×10^{-3}	1.94×10^{-3}	2.71×10^{-3}	1.46×10^{-3}
2	2.66×10^{-3}	2.68×10^{-3}	1.96×10^{-3}	1.86×10^{-3}	1.07×10^{-3}
3	3.10×10^{-3}	2.59×10^{-3}	2.00×10^{-3}	2.70×10^{-3}	1.48×10^{-3}
4	2.24×10^{-3}	2.80×10^{-3}	1.95×10^{-3}	2.58×10^{-3}	1.23×10^{-3}
5	4.43×10^{-3}	2.74×10^{-3}	2.19×10^{-3}	2.37×10^{-3}	2.25×10^{-3}
6	2.22×10^{-3}	2.72×10^{-3}	1.94×10^{-3}	2.64×10^{-3}	1.26×10^{-3}
7	2.30×10^{-3}	2.81×10^{-3}	2.35×10^{-3}	2.76×10^{-3}	1.64×10^{-3}
8	2.96×10^{-3}	2.85×10^{-3}	1.87×10^{-3}	2.83×10^{-3}	1.75×10^{-3}

Appendix 38: Malondialdehyde concentration of control donkeys transported by road during the harmattan season

Donkeys	Control donkeys				
	A Day before	After loading	After transportation	Day 3 after transportation	Day 7 after transportation
1	2.09×10^{-3}	2.85×10^{-3}	2.06×10^{-3}	2.28×10^{-3}	1.94×10^{-3}
2	2.09×10^{-3}	2.94×10^{-3}	2.03×10^{-3}	2.41×10^{-3}	2.10×10^{-3}
3	2.39×10^{-3}	2.72×10^{-3}	2.28×10^{-3}	2.14×10^{-3}	1.12×10^{-3}
4	2.72×10^{-3}	2.66×10^{-3}	1.99×10^{-3}	2.79×10^{-3}	1.70×10^{-3}
5	2.15×10^{-3}	-	2.26×10^{-3}	1.99×10^{-3}	1.24×10^{-3}

Appendix 39: Electrolytes concentrations of donkeys a day before transportation

Electrolyte	Test donkeys								Control donkeys				
	1	2	3	4	5	6	7	8	1	2	3	4	5
Na ⁺	-	126	150	141	133	139	150	133	136	146	134	144	140
K ⁺	-	14.6	20.6	22.3	15.8	14.1	16.9	8.7	19.6	14.6	9.8	17.1	16.6
Cl ⁻		92	98	108	100	102	105	94	96	96	100	102	98
HCO ₃	-	26	22	25	33	26	24	26	32	22	22	29	30
Urea	-	3.8	4.1	4.4	4.1	4.1	3.9	2.9	4.6	2.8	4.6	3.7	3.8

Appendix 40: Electrolytes concentrations of donkeys immediately after loading

Electrolyte	Test donkeys								Control donkeys				
	1	2	3	4	5	6	7	8	1	2	3	4	5
Na ⁺	141	126	138	142	132	140	133	138	137	133	146	137	133
K ⁺	5.2	4.2	6.0	4.8	5.2	4.8	4.7	4.7	3.8	4.0	5.2	4.7	4.6
Cl ⁻	100	90	100	106	100	100	98	100	98	100	100	98	106
HCO ₃	28	30	27	30	26	30	22	26	25	28	26	26	28
Urea	2.8	4.1	2.6	2.2	2.9	3.0	2.6	4.2	4.1	3.5	2.9	2.8	2.4

Appendix 41: Electrolytes concentrations of donkeys immediately after transportation

Electrolyte	Test donkeys								Control donkeys				
	1	2	3	4	5	6	7	8	1	2	3	4	5
Na ⁺	136	136	139	135	127	120	137	132	134	136	134	136	138
K ⁺	3.8	4.0	4.5	4.1	2.5	2.8	2.9	3.1	3.3	3.9	3.5	4.0	3.9
Cl ⁻	108	94	106	102	96	105	90	102	98	100	96	100	100
HCO ₃	24	20	22	24	24	18	16	22	28	24	20	18	20
Urea	3.6	3.3	4.1	2.8	2.5	2.6	3.2	4.1	3.6	4.1	3.0	4.1	3.1

Appendix 42: Electrolytes concentrations of donkeys day 3 after transportation

Electrolyte	Test donkeys								Control donkeys				
	1	2	3	4	5	6	7	8	1	2	3	4	5
Na ⁺	140	136	144	136	146	135	136	130	131	130	130	146	129
K ⁺	4.8	4.4	5.4	3.7	4.9	4.0	4.5	3.6	4.2	3.4	4.2	4.5	4.0
Cl ⁻	98	96	100	102	98	100	98	92	94	96	105	100	94
HCO ₃	25	24	30	22	25	30	26	24	27	28	18	27	28
Urea	4.5	4.1	3.6	3.2	2.8	4.1	2.6	2.8	3.1	3.3	2.8	4.1	3.3

Appendix 43: Electrolytes concentrations of donkeys day 7 after transportation

Electrolyte	Test donkeys								Control donkeys				
	1	2	3	4	5	6	7	8	1	2	3	4	5
Na ⁺	137	142	140	137	137	144	136	129	137	132	136	133	130
K ⁺	3.3	4.5	3.8	4.5	4.1	4.3	3.5	4.2	3.0	3.8	3.4	3.4	3.9
Cl ⁻	98	98	98	96	98	102	96	98	98	96	100	96	92
HCO ₃	22	22	24	32	22	28	24	25	26	30	28	33	26
Urea	2.6	3.0	3.1	2.6	3.3	2.2	2.4	2.6	2.8	4.0	2.2	2.5	2.8