EFFECTS OF PACKING ON PHYSIOLOGICAL RESPONSES AND BIOMARKERS OF OXIDATIVE STRESS IN THE ONE-HUMPED CAMEL (*Camelus dromedarius*) DURING THE HARMATTAN SEASON

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DECLARATION

I declare that the work in this thesis, entitled 'Effects of packing on physiological responses and biomarkers of oxidative stress in the one-humped camel (*Camelus dromedarius*) during the harmattan season' has been performed by me in the Department of Veterinary Physiology and Pharmacology, under the supervision of Prof J. O. Ayo, Prof P. I. Rekwot and Dr T. Aluwong. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has previously been submitted for a degree or diploma at any university.

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CERTIFICATION

This thesis entitled "EFFECTS OF PACKING ON PHYSIOLOGICAL RESPONSES AND BIOMARKERS OF OXIDATIVE STRESS IN THE ONE-HUMPED CAMEL *(Camelus dromedarius)* DURING THE HARMATTAN SEASON" by Adakole Sylvanus ADAH meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to God Almighty, my mother, beloved wife, and the scientific research community and to all men of goodwill.

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ABSTRACT

Experiments were performed in order to determine the effects of packing on physiological changes and some biomarkers of oxidative stress in the one-humped camel (*Camelus dromedarius*) during the harmattan season in the Sahel Savannah zone of Nigeria. Experimental camels (n = 8) were packed with grains of 200 kg, while control camels (n = 4) only trekked but were not packed. Ambient temperature (AT) and relative humidity (RH) of the experimental site were recorded three days prior to the experiment and on the experimental day at 06.00 h, 12.00 h and 15.00 h. Physiological parameters of rectal temperature (RT), respiratory rate (RR) and heart rate (HR) were taken before and after packing or trekking the camels. Haematological parameters and some biochemical parameters were also determined in all the camels. The AT and RH values ranged between 22.00-36.50 $^{\circ}$C and 26.00-36.00 %, respectively, and the values were outside the thermonuetral zone in the camel. The AT values which increased with the hour of the day were significantly ($P < 0.05$) and positively correlated ($r = 0.932$, 0.338 and 0.348) respectively with RT, HR and RR in both experimental and control camels. The overall mean RT recorded in experimental camels prior to packing (38.06 ± 0.20 $^{\circ}$C) was not significantly different from the value recorded after packing (38.23 ± 0.20 $^{\circ}$C). The overall mean value of RR after packing (19.09 ± 1.20 breaths/min) was significantly higher ($P < 0.05$) than overall mean RR prior to packing (16.75 ± 0.70 breaths/min). The overall mean HR after packing (51.46 ± 0.80 beats/min) was also significantly higher ($P < 0.05$) than the value obtained before packing (50.13 ± 0.40 beats/min). There were no significant differences ($P > 0.05$) in erythrocytes counts, packed cell volume, haemoglobin concentration, and leucocyte counts in both experimental and control camels after packing or trekking. The plasma total protein value was significantly higher ($P < 0.05$) in experimental camels after the packing. Following packing, the

neutrophil/lymphocyte (N/L) ratios of both experimental and control camels were not significantly different ($P > 0.05$). Concentrations of malondialdehyde (MDA), activity of superoxide dismutase (SOD) and levels of creatine were not significantly different ($P > 0.05$) following packing in both control and experimental camels. The activity of catalase, however, increased significantly in both experimental and control camels after packing and trekking. After packing, there was a significant increase ($P < 0.05$) in erythrocytes osmotic fragility of experimental camels and the values obtained were significantly higher ($P < 0.05$) than those of control camels. It is concluded that packing and trekking elevated the levels of some of the biomarkers of oxidative stress and some physiological parameters studied (HR, TP, catalase and EOF), indicating that packing and trekking were stressful to camels. It is recommended that the administration of antioxidants may be beneficial in ameliorating the adverse effects of stress due to packing or trekking in camels during the harmattan season.

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LIST OF ABBREVIATIONS

°C	Degree centigrade
AT	Ambient temperature
EOF	Erythrocyte Osmotic Fragility
FAO	Food and Agricultural Organisation
g	Grams
HR	Heart Rate
Kg	Kilograms
kcal	Kilocalories
L	Liters
m	Meters
MDA	Malondialdehyde
ml	Milliliters
mm	Millimeters
mmHg	Millimeters of mercury
mMol	Millimols
N/L ratio	Neutrophil/Lymphocyte ratio
PCV	Packed Cell Volume
RH	Relative Humidity
ROS	Reactive Oxygen Species
RR	Respiratory Rate
RT	Rectal Temperature
SOD	Superoxide Dismutase
THI	Temperature Humidity Index
TP	Total Protein

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The dromedary (one-humped) camel (*Camelus dromedarius*) is one of the most important domestic animals in the arid and semi-arid regions of the world (Yagil, 1982). It is capable of thriving, producing and reproducing in harsh climatic conditions, compared with other animals in the same habitat (Mohammed, 2008). Camels can carry loads up to 200 kg to distant places at a rate of 30 km/day (Khan *et al.,* 2003). They are comparatively cheaper means of transportation to small farmers and nomads than the conventional motorised form of transportation (Khan *et al.*, 2003). Camels are able to survive in hot temperatures that are lethal to other species (Ouajd and Kamel, 2009).

They can walk 5-7 days with little or no food and can loose a quarter of their body weights, without any impairment in normal functions (Ouajd and Kamel, 2009). In North Africa, camels are also raised for milk which has better nutritive value than cow milk. Vitamin C content in camel's milk ranges from 29-36 mg/L and is three times the level in cow milk and one-and-half times as much as in humans (Khan *et al.,* 2003; Montes, 2003). The camel meat is largely consumed in remote rural and desert areas and in cities in the Middle East (Montes, 2003).

The carcass weight of camels reared under low input varies from 180-300 kg. The dressing percentage ranges from 46–50, and the meat of a camel slaughtered at the age of 3 to 4 years tastes like beef (Khan *et al.,* 2003). They are increasingly being used for transportation of farm produce, firewood and other household goods. Camels are also used for ploughing, pulling carts and other agricultural implements and for racing (Singh, 1992).

The understanding of the physiological status of animals is necessary for their efficient control and management (Barnett and Hemsworth, 1990; Al-Haidary, 2006). It has been shown that different species of animals, and even animals of the same specie, but different genetic background, respond differently to the same stressor (Hall *et al*., 1998).

The average working hours per day in camels of both sexes ranges between 2.5 and 8 hours for pack animals and 4-6 hours for draught animals (Hanjra, 1994; Champak *et al.,* 2002). The relative draught force exerted by dromedary was about 19 % of live-weight as against 8 to 10 % for bovine, while the average working speed in the camel is 0.95 m/s (Mohammed *et al*., 1997).

It has been postulated that the camel's ability to accomplish great feats of endurance in extreme environmental conditions is due to its ability to employ unique biochemical and biomechanical mechanisms (Khan *et al*., 2003; Montes, 2003).

Stress impairs the performance of animals. It has been shown that packing of animals is stressful and may alter their physiological responses (Yousef *et al.,* 1989). Extreme temperatures cause significant stress in animals (Kataria and Kataria, 2010). Some of the physiological parameters used in the evaluation of working ability and adaptability of animals include rectal temperature (RT), heart rate (HR) and respiratory rate (RR) (Minka and Ayo, 2007; Ayo *et al.,* 2008).

Livestock experience a variety of stressors that induce generation of reactive oxygen species, which may modify normal behaviour and growth, leading to losses in performance (Nazifi *et al.,* 2009). Oxidative stress occurs when production of free radicals exceeds the antioxidative processes necessary to detoxify these toxic molecules and resulting in cellular damage and molecular disruptions (Niki, 2009). Oxidative stress plays a crucial role in the pathogenesis of various diseases, immunological incompetence and ageing (Salar-Amoli *et al.,* 2009). Formation

of reactive oxygen-nitrogen species that exceed the body's antioxidant capacity has also been termed oxidative stress (Finaud *et al.*, 2006).

Dromedaries in Nigeria are mainly found in the semi-arid northern part of the country (Mohammed, 2000). The semi-arid part of the country, which is in the Sahel Savannah zone, has the rainy season between May and September, a cold–dry (harmattan) season between October and February and a hot-dry season between March and April (Mohammed, 2000). The harmattan and hot-dry seasons have been described as thermally stressful to livestock (Igono *et al.,* 1982; Ayo and Oladele, 1996).

1.2 STATEMENT OF RESEARCH PROBLEM

In spite of the increasing number of motorized form of transportation in Nigeria, the use of camels as draught animals and beast of burdens remains irreplaceable in very remote and rural areas of the country. This is particularly true in remote areas with sandy terrain and harsh-hot climates where small-holder farm management practices are predominant.

The physiological responses which may be of value in improving the management practices of the camel in the Sahel Savannah zone of Nigeria are poorly understood. There is paucity of information on the effects of packing on the biomarkers of oxidative stress in the camel. Such information, if obtained, may enhance work output of camels in Nigeria, especially in the Sahel Savannah zone.

1.3 JUSTIFICATION FOR THE STUDY

There is an increasing use of camel for draught purposes and as a source of food. According to FAO (2009), camel population in the world is about 24 million. Camels are among the most

important animals in the arid and semi-arid regions of the world (Shafqaat e*t al*., 2004). They are kept for a variety of purposes, including transportation, racing, packing and for ploughing (Dorman, 1998).

However, data on the physiological parameters of the camel are scanty (Al-Faraj and Al-Hidary, 2006). Besides, very few studies have been carried out on the physiological and biochemical parameters of the camel subjected to packing during the harmattan season in Nigeria. Also, there is paucity of information on the impact of oxidative stress on camels and its effect on physiological parameters during the harmattan season. Such data, if available, may be of value in enhancing work output in the camel.

1.4 GENERAL AIM OF THE STUDY

The aim of the study was to evaluate the effect of stress due to packing and trekking on the physiological parameters and biomarkers of oxidative stress in the camel.

1.5 SPECIFIC OBJECTIVES OF THE STUDY

The objectives of the study were to evaluate the effects of stress due to packing on:

1. Physiological parameters of heart rate, respiratory rate and rectal temperature responses of the camel.

2. Erythrocyte counts, packed cell volume (PCV), haemoglobin concentration, total and differential leucocyte counts, erythrocyte osmotic fragility (EOF) and total plasma protein levels in the camel during the harmattan season.

3. Activities of catalase and superoxide dismutase (SOD), and levels of malondialdehyde (MDA) and creatine.

1.6 RESEARCH HYPOTHESES

The following null hypotheses (H_o) were drawn:

1. Packing does not alter the physiological responses of camels during the harmattan season.

2. Packing does not change biomarkers of oxidative stress in camels during the harmattan season in the Sahel Savannah zone of Nigeria.

CHAPTER 2

LITERATURE REVIEW

2.1 ORIGIN AND USE OF THE CAMEL

The dromedary camel is mainly found in the tropics of Africa, Middle East and Indian sub-content where it is of great importance to nomadic and rural communities for provision of high quality animal protein in the form of milk and meat and as a means of transportation (Eithahir *et al*, 2010). In the Arabian Gulf region, the camel gained popularity and importance in recent years as a racing animal (Khan *et al*., 2003). The camel is well adapted to the harsh environments characterized by inadequate water and vegetation as well as high ambient temperature and rough terrain (Eithahir *et al*., 2010).

There are 24,246,291 one-humped camels in the world (FAO, 2009), with 80 % of them in Africa. Camels are increasingly being used for the transportation of farm produce, firewood and other household goods (Kataria *et al*., 2007). Camels are also raised for milk and meat. Meat from young male camels is preferred because it tastes exactly like beef (Montes, 2003). Camel's milk is much more nutritious than that from the cow. It is lower in fat and lactose, and higher in potassium, iron and vitamin C (Khan *et al*., 2003; Montes, 2003). In many parts of Africa and Asia, camels are used to pull ploughs, turn waterwheels and transport people and goods along desert routes impassable by wheeled vehicles (Montes, 2003). The camel also produces wool, hair and hides (Khan *et al*., 2003).

2.2 MORPHOLOGY OF THE CAMEL

The dromedary or one-humped camel is a large special ruminant characterized by a long, fine, low set neck and a hump of a varying size over the thoracolumbar region (Wilson, 1984b; Khan *et al*., 2003).

At maturity, the body weight and height at the withers, respectively are 400–700 kg and 2.2-2.5 m (Wilson, 1984b). The head is carried high in an "aloof" style with anterior nasal plane almost horizontal (Wilson, 1984b). The ears are small and roundish and the eyes which are calf-like in appearance have eyelids equipped with relatively long lashes (Wilson, 1984b; Khan *et al.*, 2003).

The body has a deep, narrow chest and a relatively small abdominal waist and the croup slopes at 45° or more to the horizontal (Wilson, 1984a; Khan *et al*., 2003; Montes, 2003). The tail hardly reach the stifle and has about 8cm long hair that are restricted to the sides and tip of the tail and the udder which is four quartered is situated between the hind legs (Khan *et al*., 2003). The scrotum is situated high against the perineum and the preputial opening is directed backwards when the penis is relaxed (Wilson, 1984b; Khan *et al*., 2003).

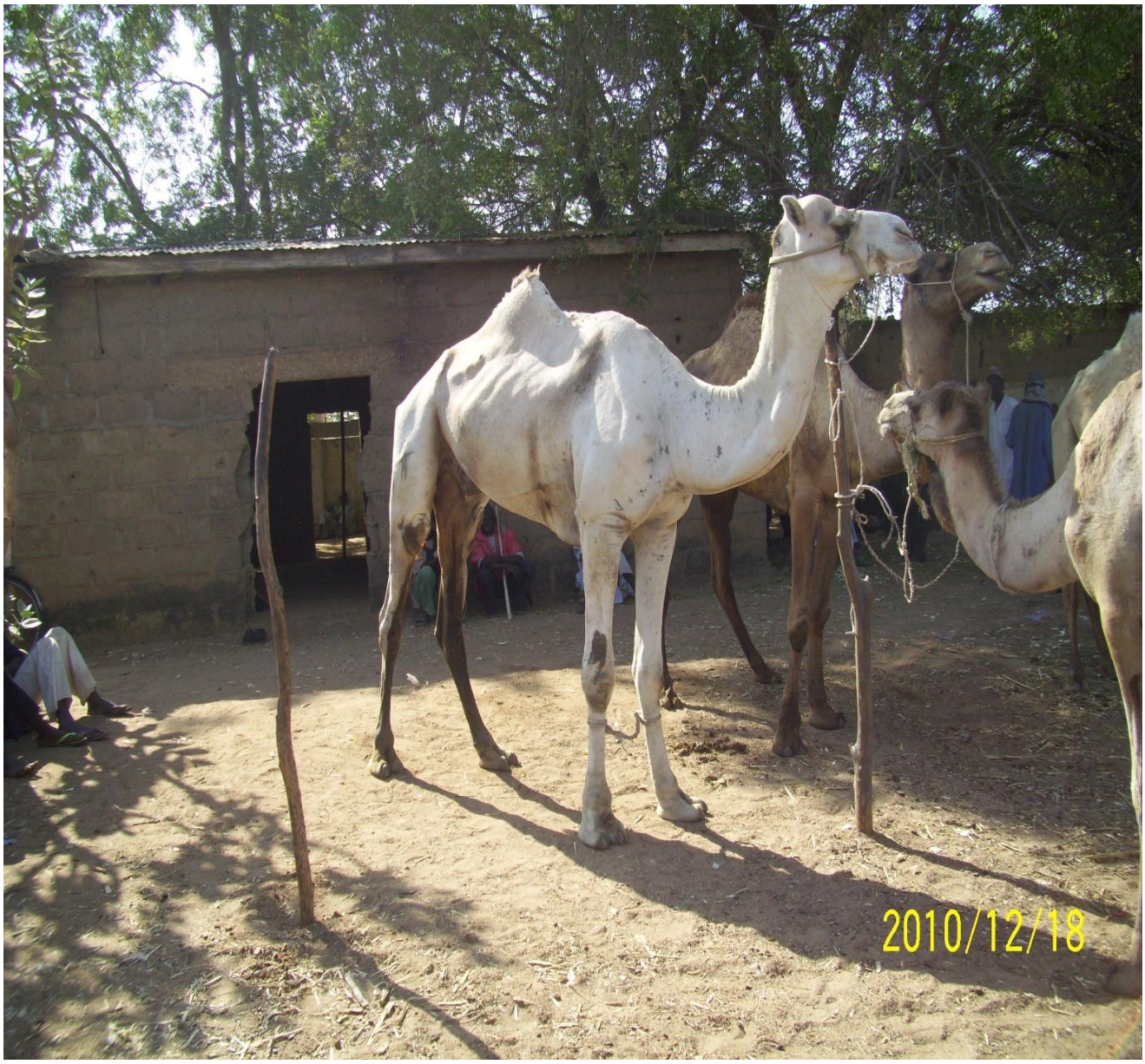


Plate I: Showing the external features of the camel



Plate II: Showing a camel packed with bags of grain

2.3 PHYSIOLOGICAL PECULIARITIES OF THE CAMEL

The dromedary is known for its ability to tolerate prolonged water deprivation and to survive in harsh desert climatic conditions (Gaithier–Pilters and Innis, 1981; Mohammed, 2008). It can survive under hot temperatures that is lethal to other species, can walk 5–7 days with little food and water and can lose a quarter of its body weights, without impairment in normal functions (Ouajd and Kamel, 2009).

Camels are homothermic animals. However, over a wide range of environmental conditions, body temperatures of camels were found to fluctuate by a magnitude of 6 $^{\circ}$C (Schimidt–Nielsen *et al*., 1957). It was reported that the rectal temperature of healthy camels at rest can vary from 34 $^{\circ}$C to more than 40 $^{\circ}$C during the summer, while diurnal changes in winter are in the magnitude of 2 $^{\circ}$C. In dehydrated state, when considerations of energy and water conservation become important, temperature variations can be as high as 8 $^{\circ}$C in the range of 34–42 $^{\circ}$C (Fazil, 1977; Wilson, 1998).

Temperature ranges are much wider in camels that are dehydrated than in camels watered daily. Also the size of fluctuation will vary with individual camel and with the climate, season and degree of dehydration (Dorqes *et al.*, 2003). The phenomenon of body temperature fluctuations assists in water conservation and is particularly important for camels reared under desert conditions, where ambient temperature may reach 50 ^{o}C or more in summer. A temperature range of 6 $^{\circ}$C in a 500 kg camel enable 1260 KJ of energy to be stored, equivalent to conservation of about 6 L of water, if sweat had to be use to dissipate the same amount of heat (Manefield and Tinson, 1996). Oxygen uptake in camel is relatively low. Maximum oxygen uptake in the camel has been recorded as 55 to 65 ml/kg/min (Khan *et al.*, 2003). Exposure of

animals to high environmental temperature stimulates the peripheral thermal receptors to transmit suppressive nerve impulses to appetite centre in the hypothalamus, causing a decrease in feed intake. Thus, less substrate becomes available for enzymatic activities, hormone synthesis and heat production, which minimize thermal load (Habeeb *et al*., 1992).

2.3.1 The Circulatory System of the Camel

The camel's cardiac rate is about 50 beats/min. It has a blood pressure that ranges from 76 to 115 mmHg and a blood volume of 93 ml/kg (Ouajd and Kamel, 2009). This value is higher than those of other domestic ruminants (Montes, 2003). Camel's blood plays a principal role in adaptive mechanisms to heat and dry conditions. Leucocytes are in large proportion made up of neutrophils (approximately 50 %) whereas in other ruminants' blood, lymphocytes are dominant (Montes, 2003; Ouajd and Kamel, 2009).

The erythrocyte is ovoid, small, relatively thin, and presents a great transferring surface. The erythrocytes continue to circulate in situation of increased blood viscosity and have the capacity to change volume depending on the state of animal hydration (Mohammed and Hussein, 1999). In the case of dehydration, the erythrocyte span life normally ranges from 90 to 120 days and this constitutes a mechanism of energy and water economy in relation to erythrocyte destruction. After rapid rehydration, changes in erythrocyte shape occur in 4 hours (Ouajd and Kamel, 2009).

The haemoglobin concentration varies from 13 to 16 g/100ml, which is relatively higher compared to other domestic ruminants. It is recognized that the camel's haemoglobin has a higher affinity for oxygen (Hogin, 2000; Ouajd and Kamel, 2009). There is increased number of charged amino acid residues on the haemoglobin which results in a higher haemoglobin

hydrophilicity and a greater resistance to osmotic dehydration and rehydration (Hogin, 2000; Ouajd and Kamel, 2009).

2.3.2 Excretory System of the Camel

The camel's kidney plays a major role in the process of conserving water through increasing the osmolarity of urine. The kidney is characterized by a long looped nephron and a well-developed medulla. The kidney has a strong capacity of water reabsorption and capacity to eliminate very concentrated urine (Siebert and Macfarlane, 1971). Contrary to other mammals, the dromedary has very unique anatomical structures in the kidney, which limit considerable urea elimination in the urine (Mahmud *et al.,* 1984). The urea appears to play a significant role during dehydration in the dromedary. By osmotic effect, the urea attracts water of other medium toward the plasma (Gihad *et al*., 1989). Camels are therefore particularly well adapted to low nitrogen diets by limiting urinary excreation of urea (Ouajd and Kamel, 2009).

2.3.3 Respiratory System of the Camel

Nostrils are able to close completely, thus avoiding draining of mucous membrane, thereby limiting water losses in the upper respiratory tracts. The lungs are deprived of lobes and the diaphragm is powerful and partly ossified. Respiratory rates vary from 9 to 20 cycles/minute and respiration is abdominal (Yagil, 1985; Wilson, 1989). Under severe heat stress, the camel does not pant. The respiratory rate decreases in the dehydrated dromedary with an increase of the partial blood pressure of carbon dioxide and reduction in that of oxygen (Yagil, 1985; Wilson, 1989). When the camel is dehydrated, the nasal passages exhibit hygroscopic characteristics. The hygroscopic nasal passage will absorb water from air that passes over it (Schmidt–Nielsen *et al*.,

1981). When dry inhaled air passes over the nasal passages, water is given off, and during exhalation, water is taken up by the dry nasal passages (Schmidt–Nielsen *et al*., 1981).

2.4 DIGESTION AND METABOLISM

2.4.1 Gastric Digestion

The pre-stomach of the camel are characterized by the presence of only three compartments in comparison with true ruminants (Khan *et al*., 2003). The great digestive capacity is due to a specific and differentiated motility, a very active micro-flora, a better microbial digestion and a more competent food mixing in the pre-stomachs (Selim *et al*., 1999). Water is absorbed very slowly from the stomach and intestines allowing time for equilibration without severe osmotic problems (Ouajd and Kamel, 2009).

2.4.2 Lipid Metabolism

The ability of the dromedary to withstand lack of food is related to its ability to mobilize body reserve of fatty tissue during malnutrition and store fats during favourable periods. In the dromedary, ketogenesis is weak in any circumstance and plasma concentrations of 3–hydroxybutyrate and acetoacetate were 33 and 4 folds, respectively, which are lower in comparison to sheep (Chilliard *et al*., 1998). Cholesterol level increases in dehydrated dromedary as a consequence of hypothyroidism (Nazifi *et al*., 1999). In dehydrated dromedaries, liver lipids decrease from 13 to 2.5 %, indicating a strong mobilization of hepatic lipids. On the other hand concentrations of triglycerides and free fatty acids remain unchanged (Nazifi *et al*., 1999). The lipids of the camel's hump are composed of phospholipids and traces of triglycerides. The size of the hump limits the deposition and dispersion of fatty tissue in other parts of the body especially

the distribution of fat under the skin and thus facilitates the cutaneous dissipation of heat (Yagil, 1985; Ouajd and Kamel, 2009).

2.4.3 Glucose Metabolism

The camel's energy metabolism differs in particular from that of ruminants. The dromedary presents a normal glycemia of about 5 mmol/l, a value that can be explained by a high gluconeogenesis and a very low level of insulin (Ouajd and Kamel, 2009). Glucose urinary elimination is accompanied by enormous water losses especially when, adequate quantity of water is available as observed in diabetes. Thus, a dehydrated camel reduces water loss by maintaining hypergycemia and practically no glycosuria (Ouajd and Kamel, 2009).

2.4.4 Nitrogen Metabolism

The greater ability of the dromedary of recycling endogenous nitrogen is an advantage considering that most food available to camels in the arid areas contain a low fraction of soluble nitrogen (Emmanuel *et al.*, 1976; Ouajd and Kamel, 2009). The camel can recycle up to 90 % of blood nitrogen, in contrast to ruminants that can only recycle 10 – 30 % (Emmanuel *et al.*, 1976). Nitrogen recycling in camels increases in the case of lower protein diet or dehydration (Gihad, *et al.*, 1989).

The metabolism of urea is strongly influenced by dehydration and a remarkable increase in uremia is noted (Ouajd and Kamel, 2009). Contrary to other mammals, the dromedary has very unique anatomical structures in the kidney, which limit considerable urea elimination in the urine (Mahmud *et al.*, 1984). The urea appears to play a significant role during dehydration in the dromedary. By osmotic effect, the urea attracts water of other medium toward the plasma (Gihad *et al.*, 1989). Camels are therefore particularly well adapted to low nitrogen diets by

limiting urinary excreation of urea, hence urea supplement in the diet can cause toxicity (Ouajd and Kamel, 2009).

2.5 ADAPTIVE PHYSIOLOGY TO HEAT STRESS AND DEHYDRATION IN THE CAMEL

Thermoregulatory capacities of the dromedary are directly related to the availability of water and its hydration status. Two essential mechanisms are implicated in heat tolerance, adaptive heterotomy and selective brain cooling (Elkhawad, 1992; Schmidt–Nielsen, 1997).

2.5.1 ADPTIVE HETEROTOMY

The camel is able to fluctuate its body temperature between 34 $^{\circ}$C and 42 $^{\circ}$C, and perspiration only takes place if the body temperature reaches 42 $^{\circ}$C (Schmidt–Nielsen, 1997). The daily temperature of a hydrated camel fluctuates by only 2 $^{\circ}$C. The dehydrated camel's temperature will differ by as much as 7 $^{\circ}$C to prevent water loss through evaporation. This mechanism allows the camel to gain approximately 2,900 kcal of heat per day which corresponds to 5 L of water being saved. The heat stored during the day (which causes drastic body temperature fluctuations) is dissipated at night (Schmidt–Nielsen, 1997).

2.5.2 Selective Brain Cooling

The camel has the ability to resist intensely high body temperatures without damaging its brain (Elkhawad, 1992). The brain cooling system provides protection for the brain in extreme temperatures and allows the camel to survive at body temperature that would normally be lethal to the sensitive brain. The camel brain is several degrees lower than body temperature because arterial blood passes over the carotid rete before reaching the brain and is cooled by venous blood returning from the evaporating surface of the long nasal cavity (Elkhawad, 1992).

2.6 OXIDATIVE STRESS

2.6.1 Molecular Oxygen and Its Reactive Derivatives

Atomic oxygen is the most abundant element in the earth's crust, molecular oxygen in the atmosphere and water is required to support all forms of aerobic life (Gilbert, 1981). Oxygen atoms are fixed into various organic molecules by a variety of enzymes e.g. oxygenanses and non-enzymatic processes (Elstner, 1982). Aerobic organisms must, however, cope with the adverse effects of oxygen (Elstner, 1982).

The toxic effect of oxygen is mainly exerted by its reactive derivatives, whereas ground–state dioxygen is rather unreactive and can peacefully co-exist with organic matter (Yu, 1994). Chemical activation occurs by univalent reduction of dioxygen, i.e. addition of electrons one by one, all three intermediates of univalent reduction namely superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) are chemically reactive and biologically toxic (Yu, 1994).

Superoxide (O_2^-) is the first reduced product of ground state oxygen, capable of both oxidation and reduction. It may react to produce several other reactive species, and may undergo spontaneous or enzymatic dismutation to hydrogen peroxide. Hydrogen peroxide is not a free radical, but participates as oxidant or reductant in many cellular reactions. It is highly diffusible through membranes and aqueous compartment and may directly inactivate sensitive enzymes at a low concentration (Wolff *et al*., 1986).

The hydroxyl radical is the most powerful oxidizing species in biological systems. It will react non-specifically with any biological molecule, and this will limit it's diffusion within the cell to a distance of two molecular diameter from its site of production (Fry, 1998). No specific

scavengers of hydroxyl radicals are known, although several metabolites, such as urea and glucose, were proposed as scavengers in animal system (Fry, 1998).

The different reactive species described above will cause, to varying extents, inhibition of sensitive enzymes and lipid peroxidation. Free radicals readily attack unsaturated fatty acids, yielding lipid hydro peroxides and in the presence of metal catalysts, alkyl and peroxyl radicals that propagate chain reactions in the membranes, changing and disrupting lipid structure and membrane organization and integrity (Yu, 1994).

In addition, some aldehyde and hydrocarbons produced by lipid peroxidation, exert cytotoxic effects on animal systems (Esterbauner *et al*., 1990). Proteins exposed to hydroxyl radicals undergo typical modification, including specific amino acid alteration, polypeptide fragmentation, aggregation, denaturing and proteolysis (Wolff *et al*., 1986). Lipids and proteins are targets of various ROS, and oxidized to give a diverse array of toxic products (Gulteridge and Halliwell, 1992).

Lipids, especially polysaturated fatty acids, are sensitive to oxidation leading to lipid peroxidation, of which malondialdehyde (MDA) is the most abundant (Saleh *et al.*, 2010). The side chains of amino acid residues of proteins are also susceptible to oxidation by the action of free radicals forming protein carbonyls. Carbonated proteins tend to form high–molecular weight aggregates that are resistant to degradation and accumulate as damaged proteins (Saleh *et al*., 2010).

Stress characterized by a marked oxygen consumption, presents challenge to the antioxidant system because of increased production of reactive oxygen species. However, tissues seem to increase their antioxidant defense when exposed to a chronic activation (Piccione *et al*., 2007).

The hypothalamo–pituitary–adrenal axis, when activated by stressors such as transport, packing, trekking and handling, responds with the release of glucocorticiods and other hormones that have physiological effects (Parker *et al*., 2003). Cortisol is the principal stress hormone associated with the activation of the hypothalamo-pituitary-adrenal–axis, and it has been shown to induce pathophysiological changes in the immune, metabolic and reproductive systems.

2.6.2 Defence against Oxidative Stress

All aerobic forms of life have evolved multiple defense lines that include scavenging enzymes and non-enzymatic antioxidants (Bannister *et al*., 1987). Such multiplicity is required because reactive oxygen species (ROS) are produced in different cellular components, and because reactive oxygen species differ in diffusion, solubility and propensity to react with various biological molecules, correspondingly diverse set of defense molecules are required to act in both aqueous and membrane phases, in all cellular components, to promptly inactivate radicals as soon as they are formed (Bannister *et al*., 1987).

A sequence of detoxification steps is often required to avoid the conversion of one reactive species into a more harmful one. The most notable is the conversion of superoxide to hydrogen peroxide by superoxide dismutase (SOD). The defense systems of the body are as follows:

2.6.3 Superoxide Dismutase

Superoxide dismutase (SOD) is a metallo-enzyme that converts superoxide to hydrogen peroxide in all aerobic organisms (Hassan, 1989). This is illustrated in the following reaction:

$$2O_2^- + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2 \quad (1)$$

Because superoxide is the first product of univalent reduction of oxygen and also the first species to form in many biological systems, SOD is considered the primary defense against oxygen radicals (Bannister *et al*., 1987). SOD is the fastest enzyme known and its three-dimensional structure has been intensively studied (Getzoff *et al*., 1992). The dismutation is catalyzed by the metal ion, copper or manganese which is attracted to such sites by appropriately positioned positively charged amino acid residues. SODs are classified as MnSODs, FeSODs and Cu/ZnSODs (Getzoff *et al*., 1992).

2.6.4 Glutathione

Glutathione, the ubiquitous tripeptide effectively reduces and detoxifies many oxidant species. A decrease in glutathione pools accompanies many stress and disease conditions (Yu, 1994). Glutathione is an endogenous antioxidant which protects cells from free radicals (Agarwal and Prabakaran, 2005). It also helps to maintain exogenous antioxidants such as vitamins C and E in their reduced and active forms. Glutathione is found almost exclusively in its reduced form, since the enzyme that reverses it from its oxidized form, glutathione reductase, is active and inducible upon oxidative stress.

The ratio of reduced glutathione to oxidized glutathione within cells is used as a measure of cellular toxicity (Pastore *et al*., 2003). Glutathione is the most abundant non-thiol protein in mammalian cells. It protects plasma membranes from lipid peroxidation, scavenges superoxide and prevents oxygen formation (Agarwal and Prabakaran, 2005). A glutathione deficiency can result to infertility in males (Hansen and Deguchi, 2005).

2.6.5 Ascorbate (Vitamin C)

Ascorbate is an important antioxidant in animal system, where it was shown to react not only with hydrogen peroxide, but also with oxygen radicals, hydroxyl radicals and lipid hydroperoxides (Yu, 1994). Tappel (1977) proposed that the antioxidant synergism between vitamins C and E in animal tissue is due to reduction of tocopherol radicals by vitamin C. Vitamin C is an important chain breaking antioxidant. Vitamin C has been shown to improve the quality of spermatozoa in male animals (Lewis *et al*., 1997). Ascorbate is capable of protecting against oxidative injuries in the aqueous compartment of cell membranes and represents the first line of antioxidant defense in compartment of cell membranes and the plasma (Yeum *et al*., 2004).

2.6.7 Tocopherol (Vitamin E)

The major isomer of vitamin E is α-tocopherol, a phenolic antioxidant present in animal tissues. Being a lipid–soluble molecule, it is very important as a chain terminator of free- radical reactions that cause lipid peroxidation (Burton *et al.*, 1982).

2.6.8 Catalase

Catalase efficiently scavenges H_2O_2 (equation 2) and does not require a reducing substrate to perform the task.

$$2H_2O_2 \xrightarrow{\text{Catalase}} O_2 + H_2O_2 \qquad (2)$$

In animal cells, peroxisomal and cystolic catalase are the primary scavengers of hydrogen peroxide (Yu, 1994). H_2O_2 is a harmful by-product of many normal metabolic processes, to prevent damage; it must be converted into less dangerous substances. Catalase is used by cells to

rapidly catalyze the decomposition of H_2O_2 into less reactive oxygen and water molecule (Gaetani *et al*., 1996). Higher catalase activity in animals is indicative of higher rate of formation of H_2O_2. This is indicative of the body's response to combat oxidative stress (Kataria *et al*., 2010).

2.7 CAMELS AND OXIDATIVE STRESS

Camels live under harsh climatic conditions and most times poor quality food (Salar-Amoli *et al*., 2009). Camels have some biochemical, anatomical and physiological peculiarities which make them adapt to arid environment with poor food quality and inadequate water supply (Saleh *et al*., 2010). Oxidative stress in camels increase susceptibility to diseases and contribute to pathogenesis of diseases (Saleh *et al.,* 2010).

Camels are used for transportation of people and goods, for racing and for ploughing and are therefore being subjected to stress of trekking and packing. Stressful conditions like transportation, trekking, packing and exercise increase oxygen uptake by the whole body particularly the skeletal muscle (Piccione *et al*., 2007). The production of reactive oxygen species (ROS) is believed to be the underlying mechanism for a series of biochemical and physiological changes that occur during stressful conditions and are indicative of oxidative stress (Piccione *et al*., 2007).

Studies done in thoroughbred horses showed that stressful conditions like transportation, packing and trekking give rise to an imbalance between free radical production and antioxidant agents, leading to oxidative stress (Sies, 1991). This stress can provide damage in several bio molecules (Natta *et al*., 1992; Brent and Rumack, 1993), creating metabolic alterations affecting physical performance. The increased free radical production, identified as semiquienone, coincides with a

series of cellular disorders such as lipid peroxidation, loss of sercoplasmatic reticulum latency and mitochondrial uncoupling (Piccione *et al*., 2007). Chronic stressful conditions have been shown to have dual effects. It induces antioxidant enzymes and perhaps stimulates glutathione peroxidase synthesis, thus theoretically facilitating the removal of reactive oxygen species produced during stressful activities (Piccione *et al*., 2007).

2.8 CORTISOL AND PROLACTIN AS INDICATORS OF STRESS

Stressful conditions and extreme temperatures as seen during harmattan and hot-dry seasons cause significant stress to livestock (Kataria and Kataria, 2010b). There are many measurable parameters of stress and cortisol hormone is one of the principal indicators of stress. Cortisol is released from the adrenal cortex in response to stress and it is responsible for many stress related physiological changes in the body (Nazifi *et al.*, 1999).

High and prolonged levels of cortisol in the body have been shown to have negative effects on the body leading to a condition of chronic stress. Research now indicates that prolactin hormone, released from the anterior pituitary, may also be an indicator of stress in animals (Kataria and Kataria, 2010b). Stress induced prolactin secretion (in animals and humans signifies its role in general adaptation syndrome (Fava and Guaraldi, 1989) and it plays a role in the management of stress (Parker *et al.*, 2003). Prolactin is now being used as a stress marker in humans (Sobrinho, 2003) and animals (Kataria and Kataria, 2010).

2.9 THERMAL ENVIRONMENTAL CONDITIONS

It has been shown that meteorological stressors that directly and adversely affect livestock performance and health are ambient temperature, relative humidity, thermal radiation and airspeed (Bianca, 1976). The overriding environmental factor affecting the body functions of

domestic animals is temperature. In a tropical country like Nigeria, animals are often under heat stress (Vathana *et al*., 2002). When the temperature falls outside the comfort zone, other climatic factors assume greater significance; humidity becomes increasingly important as do solar radiation and wind velocity (Ayo *et al*., 2008). In hot–humid climate, the ability of the air to absorb additional moisture is limited, and the inadequate cooling may result to heat stress (Vathana *et al*., 2002). The heat load on a grazing animal can be considerably increased by direct solar radiation and radiation reflected from clouds or ground (Ayo *et al*., 2008). Air movements assist in heat loss by evaporation and by conduction or convection as long as the air temperature is lower than the skin temperature (Ayo *et al*., 2008). High ambient temperature initiates compensatory and adaptive mechanisms to re-establish the body homeostasis, including homeothermy (Stott, 1981). Thus, physiological responses of the animal during exposure to environmental stress have been determined by variations in body temperature, respiratory rate and heart rate (Finch *et al*., 1982; Ayo *et al*., 1998a;Minka and Ayo, 2007a; Minka and Ayo, 2007c; Ayo *et al*., 2008). Changes in behavioural activities of animals are often the main sign of distress and the first sign of disease (Ayo *et al*., 2002).

2.10 RESPIRATION AND HEAT STRESS

Respiratory rate can be an indicator of heat stress (Habeeb *et al*., 1992). In mammals, respiration is directed at the elimination of carbon dioxide from the tissues of the body and provision of oxygen under thermonuetral condition, and evaporation of moisture from the respiratory tract and prevention of hypothermia under high ambient temperature (Marai, *et al*., 2006). Heat stress during summer is characterized by increase in respiratory rate and when a load of high relative humidity was superimposed upon an already high ambient temperature, there is further increase in respiratory rate (Marai *et al*., 2002).

2.11 RECTAL TEMPERATURE

Rectal temperature is often used as a representative measurement of animal core temperature (Nielsen, 1995). Exposure to heat stress is registered by the temperature–humidity index that includes both ambient temperature and relative humidity (LPHSI, 1990; Marai *et al*., 2001). Generally, most homeotherms maintain their body heat balance by dissipation of the excess heat from their bodies, when exposed to elevated temperatures. This is accompanied by many biological functions, of which the increase of respiration rate or panting is the most obvious reaction. Under such conditions, the animal's body temperature increases when the body fails to maintain its heat balance (Marai *et al*., 2006).

2.12 HEART RATE

The heart rate reflects primarily the homeostasis of circulation along with the general metabolic status. The rate increases on exposure to high environmental temperature (Marai *et al*., 2006). This increases blood flow from the core to the body surface to give a chance for more heat to be lost through conduction, convention and radiation and through insensible (loss by diffusion of water from the skin) means. Tanvguchi *et al*. (2004) reported hepatic blood flow to tend to be greater at 35 $^{\circ}$C than 20 $^{\circ}$C. The absorption of acetate and propionate, as well as oxygen uptake by the portal drained viscera was significantly higher at 35 $^{\circ}$C than 20 $^{\circ}$C. Generally, in wide range of environmental temperatures that comprise the thermo-neutral zone, animals maintain their heat balance via vasomotor control by regulating the amount of blood flowing through the cutaneous vessel, by either vasodilation or vasoconstriction (Marai *et al*., 2006). Vasodilation stimulates the pilomotor centre to flatten the hair cover to allow better heat dissipation through conduction, convection and radiation. As ambient temperature increases, the pulse rate, as well

as the circulation of blood increases to transfer heat from the core to the periphery (Marai *et al*., 2006).

2.13 HEAT SYNDROME

Exposure of an animal to high environmental temperature stimulates the peripheral thermal receptors to transmit suppressive nerve impulses to the appetite centre in the hypothalamus causing a decrease in feed intake. Thus, less substrate becomes available for enzymatic activities, hormone synthesis and heat production which minimizes the thermal load (Habeeb *et al*, 1992). Exposure to more heat suppresses the production of hormone-releasing factors from the hypothalamus causing an increase in pituitary hormone secretion e.g. insulin (Marai, *et al*., 2006).

The metabolic pathways slow down, causing a drastic impairment of protein utilization. This is due to dramatic decrease in apparent digestibility, volatile fatty acid production, rumen pH and electrolyte concentration in rumen fluid and a shortage in energy substrates, hormones and enzymes (Habeeb *et al*., 1992). Under these conditions, protein synthesis becomes unable to counteract the protein catabolism which leads to a negative nitrogen balance. Such destruction of protein tissue is due to increase in glucocorticoid hormones (Chandresena *et al.*, 1979; Marai *et al*., 2006).

Exposure to high ambient temperature is a major constraint in animal production as it evokes a series of drastic changes in the animal's biological functions, which include the decrease in feed intake and utilization of feed, disturbance in metabolism of water, protein, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites. Such changes result in the impairment of production and reproduction performance (Marai *et al*., 2006). The effect of

heat stress is accompanied by high humidity. Stress is manifested by activation of the neuro-endocrine system, particularly the hypothalamo-pituitary-adrenal system (Pando and Sassone–Corsi, 2001). Consequently, glucocorticoids and catecholamines are released, leading to suppression of humoral response and inhibition of the cell-mediated response (Ayo *et al.*, 1999; Speer *et al*., 2001)

2.14 THE ROLE OF SUPRACHIASMATIC NUCLEUS IN THE REGULATION OF BODY TEMPERATURE

The hypothalamus, in particular the suprachiasmatic nucleus, is an important structure regulating the circadian and seasonal rhythms of most biological functions, particularly reproductive function and behaviour in mammals (Pando and Sassone–Corsi, 2001), including the phasic and tonic release of hormones, oestrus and in some cases gonadal size (Buijs *et al*., 2003). This is due to the fact that there is evidence suggesting that the suprachiasmatic nucleus is sensitive to changes in ambient temperature, with some cells being more responsive to cold and others more responsive to heat, although photo-periodic variation may also have strong influence (Burgoon and Bailant, 2001).

The suprachiasmatic nucleus is sensitive to changes in ambient temperature and may be an important structure regulating the circadian and seasonal rhythms of most biological functions (Marai *et al*., 2006). The camel's core body temperature increases slightly during exposure to heat stress due to the fact that they dissipate excess body heat to the surrounding environment to maintain normal body temperature (Al–Haidary, 2005). Evaporative heat loss from the respiratory tract and from the skin is the main avenues of heat loss in camels. Lack of water for prolonged periods might hamper the animal's ability to dissipate heat, resulting in an increase in body temperature and hyperthermia (Al-Haidary, 2005).

2.15 EFFECTS OF HEAT STRESS ON SOME HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN CAMELS

Exposure to summer heat stress was associated with significant increases in erythrocyte counts, haemoglobin concentration, packed cell volume, albumin and globulin. The increase in packed cell volume, haemoglobin concentration and erythrocyte counts during summer might be due to haemo-concentration (Al-Haidary, 2006). Plasma total protein level does not show any significant change between summer and winter while albumin and globulin levels were significantly higher during exposure to summer heat stress. Albumin has water extracting and holding property and the higher value in camel probably indicates specific adaptation for the desert environment (Al-Haidary, 2006). Shaffer *et al.* (1981) suggested that the environment which accelerates respiratory rate would enhance rapid utilization of glucose by the respiratory muscle resulting in a decline in blood glucose level at high temperature. Tripathi *et al.* (1987) reported increased secretion of adrenaline during thermal stress causing increased glycolysis, which may be one of the reasons for the decline in glucose level at high temperature. The value of PCV was significantly higher in summer than winter (Ghosal *et al*., 1973). The increase may be due to the camel's response to higher ambient temperature for better oxygen carrying capacity (Al-Haidary, 2006).

CHAPTER 3

MATERIALS AND METHODS

3.1 MANAGEMENT OF CAMELS

A total of 12 apparently healthy adult male dromedary camels were used for the study. They were dewormed using albendazole (Pharma Swede, Egypt) and screened for haemoparasitic diseases and only healthy camels served as subjects. The camels were preconditioned for two weeks before the experiment. They were divided into two groups. Group I consisted of eight camels which constituted the experimental group and group II comprised of four camels which served as the control. The camels were raised under semi-intensive system and their feed was supplemented with hay. Water was provided *ad libitum*. The experiment was carried out in Kasuwar-daji ($12^{\circ}36'$ N, $6^{\circ}35'$ E), Zamfara State, located in the Sahel Savannah zone of Nigeria.

3.2 LIVE WEIGHT MEASUREMENT

The body weight of each camel was determined approximately using a weight measuring tape (The Kruse Company, Denmark). The live weights were calculated using the formula of Khan *et al.* (2003) as shown below:

$$\text{Live weight, (kg)} = \text{Shoulder height (m)} \times \text{Thoracic girth (m)} \times \text{Hump girth (m)} \times 50.$$

3.3 MEASUREMENTS OF RECTAL TEMPERATURE (RT), RESPIRATORY RATE (RR) AND HEART RATE (HR)

The RT, HR and RR rates of each camel were determined and recorded before and after the experiment. The RT was taken using a digital clinical thermometer (The Hartman's Company, England). The thermometer was inserted into the rectum of each animal and the RT value was read after a beep sound was heard, indicating the end of the reading. The HR was taken by

auscultation of the heart using a stethoscope, and counting the number of heart beats per minute. RR was determined by counting the number of respiratory flank movements per minute as described by Zaytsev *et al*., 1971 and Mohammed *et al.*, 2007.

3.4 MEASUREMENT OF THERMAL ENVIRONMENTAL CONDITIONS

The dry-and wet-bulb thermometer (Brannan, England) was used to measure relative humidity and dry-bulb and wet-bulb temperature of the experimental site at 06.00 h, 12.00 h and at 15.00 h. The relative humidity was calculated using the instructions of the manufacturer. Temperature humidity index (THI) was calculated according to the formula of Al-Haidary (2006):

$$THI = Td – (0.55 – 0.55 \times RH) (Td - 58)$$

Where Td = dry-bulb temperature in Fahrenheit

RH = relative humidity in decimals

3.5 PACKING OF CAMELS

Each camel in the experimental group was loaded with bags of grain weighing 200 kg as described by Khan *et al*. (2003). The weight of the bags of grain was measured using a weighing scale (Philips Harris, England) RT, RR and HR were determined from each camel using standard methods and blood samples were collected before and after trekking a distance of 10 km.

3.6 BLOOD SAMPLING

Blood samples (10 ml) were obtained from each camel before and at the end of the experiment. The samples were collected by jugular venipuncture into vacuum containers, containing the anticoagulant, ethylenediaminetetraacetic acid (EDTA) and into containers without EDTA. The blood samples were transported to the Physiology Research Laboratory of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria in a Coleman box for analyses. Samples were analysed for the following parameters: erythrocyte counts, packed cell volume (PCV), haemoglobin concentration, total and differential leucocyte count, total plasma protein and erythrocyte osmotic fragility and serum biochemical parameters including malondialdehyde, creatine and activities of superoxide dismutase and catalase.

3.7 HAEMATOLOGICAL PARAMETERS

Blood samples containing EDTA were aspirated into plain capillary tubes and centrifuged at 750 × *g* for 15 minutes. Thereafter, PCV of each sample was read using the haematocrit graphic reader (Kelly, 1984). The haemoglobin concentration of each sample was determined using cyanmethaemoglobin method (Kelly, 1984). The total protein of the samples was measured using the hand refractometer as described by Benjamin (1985). Total and differential leucocyte counts were also determined using a standard Haematology Analyzer (Abbott Laboratories, Abbott Park, Illinois, U.S.A.). Erythrocyte count was determined using haemocytometer (Schalm *et al*., 1975).

3.7.1 ERYTHROCYTE OSMOTIC FRAGILITY (EOF)

The EOF of the samples was determined using the procedure described by Amin *et al.* (2007). Briefly, phosphate-buffered sodium chloride stock solutions (pH 7.4) were prepared in volume of 500 ml for each of the sample in 5 different concentrations of 0.1 %, 0.3 %, 0.5 %, 0.7 % and 0.9 %. A set of 5 test tubes was used and each tube contained 5 ml of the corresponding NaCl concentration from the stock solution. Exactly 0.02 ml of each blood sample was added to each of the 5 test tubes. The contents of the test tubes were mixed gently by inverting the test tubes about 5 times and allowing them to stand at room temperature (25 ºC) for 30 minutes. Thereafter, the contents of the test tube were centrifuged at 1500 × *g* for 15 minutes. The supernatant was transferred into a glass cuvette and the optical density measured at a wavelength of 540 nm using a spectrophotometer (Spectronic-20, Philip Harris Limited, Shenstone, UK), by reading the absorbance. The percentage of haemolysis was calculated according to Fraukner and King (1970), as follows:

Percentage haemolysis = Optical density of test/Optical density of standard (distilled water) × 100 %

3.7.2 Evaluation of Serum Biochemical Parameters

Blood samples for analyses of serum biochemical parameters were collected in bottles (without anticoagulant) and allowed to clot. The clotted blood samples were centrifuged at 1000 × *g* for 10 minutes, and the serum samples obtained were evaluated for creatine as described by Bogin (2000), catalase according to method of Kataria *et al*. (2010a), superoxide dismutase as described by Nazifi *et al*. (2009) and malondialdehyde using the method of Draper and Hadley (1990).

3.7.3 Determination of Serum Malondialdehyde (MDA) Concentration

Malondialdehyde concentration in the serum sample was determined by the double-heating method of Draper and Hadley (1990). The method was used to measure the level of thiobarbituric acid (TBA) reactive substance, expressed as the extent of MDA production, an index of lipid peroxidation. The method was based on spectrophotometric measurement of the colour developed during reaction of TBA and MDA. Serum samples were centrifuged at 1000 × *g* for 10 minutes at 4 °C to sediment blood cell debris. Exactly 2.5 ml of 100 g/l trichloroacetic acid solution was added to 0.5 ml serum in a tube and placed in a boiling water (100 ºC) bath for 15 minutes. Test tubes were placed in another beaker of tap water bath for 5 minutes to cool. The mixture was centrifuged at 1000 × *g* for 10 minutes. Thereafter, 2 ml of the resultant supernatant was added to 1 ml of 6.7 g/dl thiobarbituric acid solution in a test tube and placed in a boiling water bath (100 °C) for 15 minutes. The solution was again cooled in ordinary water bath (10 ºC). After cooling, the absorbance of resulting sample was measured at 532 nm using a spectrophotometer (Spectronic-20, Philips Harris Limited® Shenstone, UK). The MDA concentration in the serum was calculated by the absorbance co-efficient of MDA-TBA complex - 1.56×10^5/cm and expressed as µmol/ml. The concentration of MDA in the serum was calculated as follows: (Draper and Hadley, 1990).

Absorbance co-efficient of MDA-TBA complex, $A = ecl$

Where:

A = Absorbance

L = length which is equal to 1

C = Concentration

Therefore, c = A/el = A/e

e = 1.56×10^5 /cm/M

c = A/1.56×10^5 (mol/L) (x 10^9/ 10^3 to get answers in nMol/ml.

3.8 STATISTICAL ANALYSIS

Data obtained from the study were expressed as mean ± standard error of the mean (Mean ± SEM) and analysed using the Student's *t*-test to compare the differences in the mean values between the control and experimental camels. Values of $P < 0.05$ were considered significant.

CHAPTER 4

RESULTS

4.1 THERMAL ENVIRONMENTAL CONDITIONS

The thermal environmental conditions from the study period are shown in Table 4.1. The dry bulb temperature had minimum and maximum values of 22 ± 0.70 °C and 36.20 ± 0.20 °C, respectively with overall mean of 31.30 ± 4.70 °C. The relative humidity (RH) ranged between 26 - 36 %, while the ambient temperature (AT) was between 22-36.50 °C, with range of 13.50 °C. Temperature humidity index (THI) had a minimum value of 66.16 ± 0.50 and a maximum value of 83.88 with an overall mean of 77.16.

4.2 RECTAL TEMPERATURE, HEART RATE AND RESPIRATORY RATE OF CONTROL AND EXPERIMENTAL CAMELS BEFORE EXPERIMENT.

The rectal temperatures (RT) of the experimental and control camels prior to packing are shown in Table 4 2. The minimum and maximum mean values of RT of the experimental camels were 37.79 ± 0.20 °C at 06.00 h and 38.34 ± 0.30 °C at 12.00 h, respectively. The rectal temperatures of the control camels had a minimum mean value of 37.58 ± 0.20 °C and a maximum value of 37.88 ± 0.60 °C. Values of RT obtained were not significantly different ($P > 0.05$). The heart rates of the experimental and control camels are shown in Table 4.3. The minimum value of the HR of the control camels was 48.00 ± 1.30 beats/min while the maximum value was 50.50 ± 0.60 beats/min. The minimum value of HR obtained for the experimental camels was 49.63± 0.80 beats/ min and the maximum value was 51.00 ± 1.00 beats/min. All values obtained were not significantly different ($P > 0.05$).

Table 4.4 shows the respiratory rate (RR) of the control and experimental camels prior to packing. The control camels had a minimum value of 17.00 ± 1.30 breaths/min and a maximum value of 20.25 ± 0.90 breaths/min. The experimental camels had a minimum value of 14.88 ± 0.80 breaths/min and a maximum value of 17.75 ± 0.90 breaths/min. Values obtained for minimum or maximum HR were not significantly different ($P > 0.05$) between the two groups.

4.3 RECTAL TEMPERATURE, HEART RATE AND RESPIRATORY RATES OF CONTROL AND EXPERIMENTAL CAMELS AFTER PACKING.

Table 4.5 shows the effect of packing on rectal temperatures of the camels. The minimum value for control camel was 37.55 ± 0.20 °C at 12.00 h and a maximum value of 38.20 ± 0.40 °C at 15.00 h with overall mean of 37.89 ± 0.20 °C. The minimum value of rectal temperature for experimental camel was 38.04 ± 0.30 °C at 12.00 h and a maximum value of 38.60 ± 0.40 °C at 15.00 h with overall mean of 38.23 ± 0.20 °C. Values obtained were not significantly different ($P > 0.05$) between the groups.

Table 4.6 shows the effects of packing on heart rates (HR) of camels after the experiment. The control at 15.00 h camels had a minimum value of 48.50 ± 1.80 beats/min and a maximum value of 50.50 ± 1.8 beats/min. The experimental camels had a minimum value of 50.13 ± 0.70 beats/min at 12.00 h and a maximum value of 52.88 ± 0.60 beats/min at 09.00. The overall mean heart rate of experimental camel (51.46 ± 0.80 beats/min) was significantly higher ($P < 0.01$) than overall mean heart rate (49.25 ± 0.60 beats/min) of control camels.

Table 4.7 shows the effects of packing on respiratory rates of camels. The minimum value for the control camel was 17.00 ± 1.30 breaths/min at 12.00 h and a maximum value of 20.75 ± 1.00 breaths/min at 09.00 h, with overall mean of 18.50 ± 1.10 breaths/min. The minimum value of the experimental camel was 17.38 ± 0.6 breaths/min at 12.00 h and a maximum value of 21.50 ± 0.90 breaths/min at 09.00 h with overall mean of 19.09 ± 1.20 breaths/min. There was no significant difference ($P > 0.05$) between the overall mean RT of control and experimental camels.

Hour of the day, AT and RH values were positively correlated with RT, RR and HR values in both experimental and control camels before packing and trekking (Tables 4.8 and 4.9).

4.4 HAEMATOLOGICAL PARAMETERS OF CAMELS

The Packed cell volume (PCV) and haemoglobin concentration (Hb) values of experimental camels prior to the packing (29.75 ± 6 % and 7.44 ± 0.19 g %) were not significantly different ($P > 0.05$) from corresponding values obtained after experiment ($P < 0.05$) (31.75 ± 0.84 % and 7.81 ± 0.2 g%) (Table 4.10). The total protein of the experimental camel prior to packing (71.63 ± 1.80 g/l) was significantly lower ($P < 0.05$) than the value obtained after packing (78.88 ± 1.90 g/l). The leucocyte counts of the experimental camel prior to packing (18.66 ± 0.4 x 10^9/L) were not significantly different ($P > 0.05$) from values obtained after packing (19.66 ± 0.63 ×10^9/L). The values of differential leucocyte counts of neutrophils, lymphocytes, eosinophils, monocytes, basophils and band cells obtained prior packing were not significantly different ($P > 0.05$) from corresponding values obtained after packing (Table 4.10).

The neutrophil/lymphocyte (N/L) ratios were not significantly different ($P > 0.05$) before and after packing in experimental camels (Table 4.10). PCV of control camels before and after packing were also not significantly different ($P > 0.05$). Values obtained were 25.71 ± 1.1 % and 26.05 ± 1.10 % respectively (Table 4.11). The values of Hb of control camels prior to trekking were not significantly different ($P > 0.05$) from value obtained after trekking. Values obtained were 7.33 ± 0.35 g% and 7.53 ± 0.33 g% respectively. Total protein value of control camels before trekking (71.630 ± 1.80 g/l) was not significantly different ($P > 0.05$) from value obtained after trekking (73.00 ± 2.20 g/l) (Table 4.11).

Total leucocyte counts of control camels before trekking (17.00 ± 0.5 × 10^9/L) were not significantly different ($P > 0.05$) from value obtained after trekking (17.50 ± 0.61 × 10^9/L). The value of erythrocyte counts of control camels after trekking (15.30 × 10^{12}/L) were also not significantly different ($P > 0.05$) from values obtained before experiment (15.05 × 10^{12}/L) (Table 4.11). Neutrophil, lymphocyte, eosinophil, monocyte, band cell and basophil counts obtained before experiment were not significantly different ($P > 0.05$) from values obtained after trekking in control camels (Table 4.11). N/L ratio of control camels before trekking was not significantly different ($P > 0.05$) from value obtained after the trekking.

The PCV of experimental camels (31.75 ± 0.84 %) after packing was significantly higher ($P < 0.01$) than that of control camels after packing (26.05 ± 1.10 %) (Table 4.12). There was no significant difference ($P > 0.05$) between Hb concentration of experimental camels after packing

and that of the corresponding values of control after packing ($P > 0.05$). Values obtained were 7.81 ± 0.20 g% and 7.53 ± 0.33 g% respectively. The TP of experimental camels after packing (78.88 ±1.90 g/l) was significantly higher ($P < 0.05$) than corresponding value (73.00 ± 2.20 g/l) of control camels (Table 4.12). Total leucocyte counts of experimental camels (19.66 ± 0.63×10^9/L) after packing were not significantly different ($P > 0.05$) from corresponding value (17.50 ± 0.61×10^9/L) obtained in control camels. Erythrocyte counts of experimental camels were significantly lower ($P < 0.05$) than values obtained in control camels after packing (Table 4.12). Values obtained were 10.45 ± 0.33×10^9/L and 15.30 ± 0.22×10^9/L respectively. There was no significant difference ($P > 0.05$) after packing between experimental and control camels in the counts of neutrophils, lymphocytes, eosinophils, monocytes, basophils, and band cells after packing (Table 4.12). There was also no significant difference ($P > 0.05$) between N/L ratio (1.89 ± 0.09) of experimental camels and that of control camels (1.78 ± 0.04) after packing.

4.5 EFFECTS OF PACKING ON SERUM BIOCHEMICAL PARAMETERS OF CAMELS

The serum biochemical changes of experimental and control camels subjected to packing and trekking are shown in Tables 4.13-4.15. As shown in Table 4.1, there was no significant difference ($P > 0.05$) in the level of Malondialdehyde (MDA). Values obtained were 2.06 ± 0.06 nmol/ml and 2.2 ± 0.08 nmol/ml respectively. There was also no significant difference in activity of superoxide dismutase before packing (150.9 ± 4.3 U/L) and the corresponding value obtained after packing (162.1 ± 3.4 U/L) in experimental camels. The activity of the enzyme catalase increased significantly ($P < 0.01$) after packing in experimental camels from 106.9 ±2.80 U/L to 122.6 ± 3.9 U/L.

There was no significant difference (P > 0.05) in the level of creatine after packing in experimental camels (Table 4.13). Values obtained were 97.15 ± 7.01 µMol/L and 101.4 ± 7.45 µMol/L respectively. There was no significant difference (P > 0.05) in the concentration of MDA before trekking (2.07 ± 0.09 nMol/ml) and the corresponding value (2.12 ± 0.09 nMol/ml) obtained after trekking in control camels. There was also no significant difference (P > 0.05) in the activity of SOD in control camels after trekking. Values obtained were 144.4 ± 5.2 U/L and 151.5 ± 5.7 U/L respectively. The activity of catalase significantly increased (P < 0.05) from102 ± 4.36 U/L to 117.20 ± 2.56 U/L in control camels after trekking. There was no significant difference (P > 0.05) in level of creatine before trekking (100.20 ± 3.50 µmol/L) and the corresponding value obtained (101.90 ± 3.40 µmol/L) after trekking in control camels (Table 4.14).

Table 4.15 shows the serum biochemical parameters of experimental and control camels after packing. There was no significant difference (P > 0.05) between MDA values of experimental and control camels after packing. Values obtained were 2.2 ± 0.08 nMol/ml and 2.12 ± 0.09 nMol/ml for experimental and control camels respectively. There was also no significant difference (P > 0.05) in the activity of SOD (162.10 ± 3.40 U/L) in experimental camels and the corresponding value obtained in control camels (151.50 ± 5.70 U/L) after packing. There was also no significant difference (P > 0.05) in the activity of catalase (122.60 ± 3.90 U/L) obtained in experimental camel and the corresponding value (117.20 ± 2.56 U/L) obtained in the control camels after packing. There was also no significant difference (P > 0.05) in level of creatine between experimental camels and control camels after packing.

4.6 EFFECTS OF PACKING ON ERYTHROCYTE OSMOTIC FRAGILITY (EOF) OF CAMELS

Figure 4.1- 4.3 show the effects of packing on osmotic fragility of camels' erythrocytes. Fig 4.1 reveals that the fragility of erythrocytes of experimental camels increased significantly ($P < 0.05$) after packing at NaCl concentrations of 0.1%, 0.3%, 0.5% 0.7% and 0.9%. However, packing did not significantly alter the erythrocyte fragility in the control camels at the various NaCl concentrations studied (Fig 4.2). On the other hand, the erythrocyte fragility was significantly higher ($P < 0.05$) after packing in the experimental camels than in control camels at NaCl - concentrations of 0.3%, 0.5%, 0.7% and 0.9% (Fig 4.3).

CHAPTER 5

CHAPTER FIVE

DISCUSSION

The thermal environmental conditions are shown in Table 4.1. The ambient temperatures ranged between 22 and 36.50 °C, and the relative humidity varied between 22 and 36 %. These values were outside the thermonuetral zone for camel's work output. Wilson (1984a) and Khan *et al.* (2003) reported an ambient temperature range of 20-32 °C and relative humidity of 15-20 % as being favourable for camel's work output. Thus, the micro-climatic conditions under which this study was carried out during the dry harmattan in Sahel–Savannah zone of Nigeria could not be considered optimum for camel's work output and therefore could be sources of stress to the animals.

The AT from the results obtained showed diurnal fluctuations with minimum temperature of 22 °C and maximum of 36.50 °C. High ambient temperatures have been shown to be thermally stressful to camels. Prolonged exposure to ambient temperature impairs camel's ability to dissipate body heat to the environment (Schimidt-Nielsen *et al.*, 1967). The AT in this study area exhibited a circadian rhythm reaching a maximum of 36.50 °C at midday (12.00 h) and a minimum of 22 °C in the early morning (06.00 h). This agreed with the work of Al-Haidary, (2006) that such range of AT is thermally stressful to camels. The fluctuation in RH of 26-36 % is outside the thermonuetral zone of the camel whose performance has been demonstrated to improve under very low RH of 10-20 % (Wilson, 1998; Khan *et al.*, 2003). The findings show that the harmattan season in the Sahel Savannah zone of Nigeria was thermally stressful to camels in the daytime. Therefore for camels to be subjected to packing and trekking during this season, they must develop an efficient adaptive mechanism. The AT of 32.33 ± 4.2 °C obtained in this study was different from the 28 ± 2.6 °C recorded by Mohammed *et al*. (2007) in the

study on the effects of season, sex and vital parameter on the adult one-humped camel in Shika-Zaria. The differences may be due to variations in climatic conditions of the study areas. There was, however, no significant difference in the AT recorded in this study (32.33 ± 4.20 °C) and that obtained by Al-Haidary (2006) (32.00 ± 0.11 °C), who studied seasonal variation and its effects on some physiological responses in Arabian camels in the cold season in Saudi Arabia. There was also no significant difference in the RH value obtained in this study (33 ± 3.00 %) and the value of 32.51 %, obtained by Al-Haidary (2006) in his study.

The RTs of both experimental and control camels showed diurnal fluctuation. The experimental group prior to packing had a minimum RT of 37.30 °C at 06.00 h and a maximum of 39.30 °C at 15.00h. This is in agreement with the work of Al-Haidary (2006) and Mohammed *et al*. (2007) who reported lower values of RT at the early hours of the morning and a higher value at midday time. The result of this study is also in agreement with previous reports of Schimidt-Nielsen *et al*. (1957) and Wilson (1998) that the diurnal fluctuations in rectal temperatures of camels provided with water *ad libitum* is of the order of 2 °C (36.96-38.06 °C). The diurnal fluctuation is also in agreement with the findings in cattle (Ayo *et al*., 1997b), goats (Ayo *et al*., 1998b) and in donkeys (Ayo *et al.,* 2008) in Zaria, Nigeria. Schimidt-Nielson *et al*. (1967) reported that dehydrated camels are capable of fluctuating their RT in the order of 6 °C (36.2 °C – 42 °C) as a mechanism of water conservation. The wide fluctuations are largely determined by temperature of the environment, hence the need to use RT as an indicator of health status in camels with caution as suggested by Mohammed *et al*. (2007).

The RT of both experimental and control camels decreased from 38.06 ± 0.2 °C to 38.04 ± 0.3 °C and 37.93 ± 0.2 °C to 37.55 ± 0.2 °C, respectively immediately after packing and trekking at

09.00 h and 12.00 h (Table 4.2) clearly indicating the effects of stress of packing and trekking on the camels.

The heart rates of camels prior to packing also showed diurnal fluctuations. In the experimental camels, the value of 49.75 ± 0.7 beats/min was recorded at 06.00 h and rose to 51.00 ± 1.0 beats/min at 12.00 h (Table 4.3). This clearly shows the effect of AT and RH on HR of camels as high AT and RH constitute sources of stress to animals (Marai *et al*., 2006). The results obtained showed an increase in HR value from 49.75 ± 0.7 beats/min at 06.00 h to 52.88 ± 0.66 beats/min immediately after the packing in the experimental group, indicating that packing and trekking were stressful to camels. This result agrees with the finding of Zapata *et al*. (2004) in captive-reared, guanacos (*Lama guanicoe*) who reported that stress of road transportation increases the HR of the species. The result also agrees with that of Minka and Ayo (2007b), who showed that transportation stress increases HR in ostriches. The value of HR in control group also rose from, 48.00 ± 1.3 beats/min at 06.00 h to 48.75 ± 1.1 beats/min immediately after trekking. This increase in HR confirms the effect of stress of trekking on HR of the control camels, although the difference in the values was not significant. The findings of this work showed a significant difference in HR values of experimental camels immediately (15 minutes) after the packing and trekking in HR of experimental camel and those of control camel. Experimental camels had a higher HR indicating that packing exerted additional stress to the camels. The HR of the experimental camels decreased significantly ($P < 0.05$) from 52.88 ± 0.6 beats/min immediately after trekking to 50.13 ± 0.7 beats/min at 12.00. This is in agreement with the findings of Zapata *et al*. (2004), who reported a decrease in HR of captive reared guanacos during transportation to a peak value at the end of transport and a steady decline in value of HR to values recorded just before transportation. This also agrees with the findings of Smith *et al*. (1994) that HR decreased

significantly during road transport and failed to return to pre-transport values in horses. All values of HR recorded in the present study were within the normal range of 48-56 beats/min for camels (Bogin, 2000).

The RR values of camels obtained in this work also showed diurnal fluctuation, but pre-packing values of experimental group, (17.67 ± 0.7 breaths/min) at 06.00 h were significantly higher than the 14.88 ± 0.8 breaths/min recorded at 12.00 h. This may be attributed to the fact that at the early hours of the day the camels were actively ruminating. Thereafter, the value rose to 17.75 ± 0.9 breaths/min at 15.00 h, which may be due to the effects of higher AT and RH. Diurnal fluctuations in RR were also observed in sheep, (Ayo *et al*., 1997a) and donkeys, (Ayo *et al*., 2008). All values recorded in this study were within the normal RR range for camels (9-25 breaths/minute) (Ouajd and Kamel, 2009). There was a significant difference between RR values obtained immediately after trekking at 09.00 h and that of 12.00 h in the experimental and control groups with the values immediately after experiment being significantly higher than those recorded at 12.00 h after camels had rested. This finding confirmed that packing and trekking were stressful to camels. There was no significant difference ($P > 0.05$) between mean RR of control camels and that of experimental camels after packing. The finding contradicts the result of Yagil (1985) and Wilson (1998) who reported that under stressful conditions the RR of camels decreases with an increase in partial blood pressure of carbon dioxide and reduction in that of oxygen.

The results of all haematological parameters in both experimental and control groups were within the normal range for camels (Bogin, 2000). The results of haematological parameters in experimental camels showed a significant increase ($P < 0.05$) in the TP of experimental camels (Table 4.10). This may be attributed to increased activity of albumin which is a principal factor

in the maintenance of plasma hydration to prevent extracellular dehydration during stressful conditions (Ouajd and Kamel, 2009), and as a result of generation of reactive oxygen species due to stress of packing. Albumin is an endogenous antioxidant that increases to combat oxidative stress during stressful situations like packing because it contains a thiol group, which protects vital targets such as erythrocytes from copper ion-induced Fenton reaction and inhibits iron-dependent free radical production (Argawal and Prabukaran, 2005; Roche *et al.*, 2008). Similar increase in TP was reported in calves (Earley *et al*., 2006) transported by road and in sheep (Kumar *et al*., 2003) subjected to road transportation.

Total leucocyte counts were not significantly different ($P > 0.05$) after packing in both control and experimental camels. This finding does not agree with that of Zapata *et al*. (2004), who reported leucocytosis following stress of road transportation in captive-reared guanacos (*Lima guanicoes*). Species variation may be the reason for the difference. Counts of neutrophils, lymphocytes, monocytes, eosinophils, basophils, and band cells after packing were not significantly different ($P > 0.05$) from those obtained prior to packing in both experimental and control camels. The results however, contradict those of Zapata *et al*. (2004) who recorded a decrease in lymphocyte count following transport of guanacos by road. Neutrophil/lymphocyte ratio did not change significantly ($P > 0.05$) after packing and this also contradicts the result obtained by Zapata *et al*. (2004) in guanacos (*Lima guanicoes*) and that of Kock *et al*. (1999) in the black rhinoceros (*Diceros bicornis*), where neutrophil/lymphocyte ratio was significantly higher after subjecting the animals to stress of road transportation. The change recorded in TP this present study may be a compensatory mechanism adopted by the camels to reduce the adverse effects of stress of packing and trekking.

There was no significant change ($P > 0.05$) in serum MDA concentration of experimental camels after packing and trekking. MDA, which is one of the end-products of lipid peroxidation, is usually measured to evaluate the extent of lipid peroxidation (Lata *et al*., 2004). A significant increase in MDA concentrations in dromedaries was reported by Nazifi *et al*. (2009) after transportation of camels by road during summer in an opened truck in Iran. The difference in the findings may be attributed to the different climatic conditions prevailing in the two study areas.

There was no significant difference ($P > 0.05$) in the activity of SOD in both experimental and control camels before and after packing. This agrees with the finding of Nazifi *et al.* (2009), who reported that there was no significant difference in the activity of SOD following transportation of camels by road in Iran.

The activity of the enzyme catalase rose significantly ($P < 0.05$) after packing and trekking in both control and experimental camels (Tables 4.13 and 4.14). Catalase functions to catalyze the decomposition of hydrogen peroxide to water and oxygen (Chelikani *et al*., 2004). Hydrogen peroxide is a harmful by product of many metabolic processes. To prevent cellular damage, it must be quickly converted into less dangerous substances (water and oxygen). A high catalase activity is an indication of a high rate of formation of hydrogen peroxide which indicates the response of the body to oxidative stress. The high activity of catalase recorded in the present study is evidence that the body system of the camels were responding to oxidative stress. This finding confirms that packing and trekking were stressful to the camels. Excessive and continuous generation of ROS has been shown to exert harmful effects on cell resulting in loss of fertility (Agarwal and Prabakaran, 2005).

The result of erythrocyte osmotic fragility showed very little haemolysis at 0.9 %, 0.7% and 0.5% NaCl concentrations in both control and experimental camels as shown in Figures1-3. This is due to the ability of camel erythrocyte to swell up to 240 % of normal size without bursting but in other species erythrocyte can only swell up to 150 % before haemolysis will occur (Montes, 2003), These findings shows that erythrocytes of camels are very resistant to haemolysis. In this study, haemolysis began to occur at 0.3 % NaCl concentration. There was a significant increase in haemolysis ($P < 0.05$) at 0.9 %, 0.7 %, 0.5 %, 0.3 % and 0.1 % NaCl concentration, in the experimental after packing, indicating that packing was stressful to the camel. This is because packing, being stressful a condition increased ROS generation, which depleted antioxidant level of the erythrocyte membrane, resulting in haemolysis (Hanzawa and Watanabe, 2000; Romeu *et al*., 2010). In the erythrocytes, free radicals enhance oxidative damage to membrane protein principally in the form of disulphide linked haemoglobin skeletal protein adducts, causing haemoglobin denaturing and cytoskeletal instability (McMillan *et al.,* 2005). There was a significant increase in haemolysis in experimental camels than control camels as shown in Figure 3. This suggests that packing could have exerted additional stress to the camels. The results of EOF recorded in this study agreed with the finding of Amin *et al.* (2007), who demonstrated that marked haemolysis of erythrocytes of camels began only at 0.3 % NaCl concentration in the dry season.

CHAPTER 6

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY

The AT and RH values recorded pre-packing and post-packing may be outside the thermonuetral zone for work output in the camel during the harmattan season prevailing in the Sahel Savannah zone of Nigeria.

The RT, RR and HR values in experimental and control camels showed diurnal fluctuations pre-packing and post-packing.

The fluctuation in RT of camels was 2 $^{\circ}$C since they were provided with water *ad libitum.*

There was a significant increase in RT, HR, and RR immediately after the experiment in both experimental and control camels at 09.00 h and at 12.00 h indicating the effect of stress of packing and trekking.

The changes in the value of HR and in experimental camels was significantly higher than that recorded in control camels, indicating that packing could have exerted more stress to the camels.

Post-packing TP increased significantly compared to corresponding pre-packing value in experimental camels.

The activity of catalase increased significantly after packing in both experimental and control camels.

The EOF of experimental camels was significantly higher ($P < 0.05$) after packing at 0.9 %, 0.7 %, 0.5 %, 0.3 % and 0.1 % NaCl concentration. The haemolysis at 0.9 %, 0.7 % and 0.5 % NaCl concentration were not significantly different ($P > 0.05$) in the control camels. EOF was significantly higher ($P < 0.05$) at 0.3 %, 0.5 %, 0.7 % and 0.9 % NaCl concentration in experimental camels than control camels.

6.2 CONCLUSIONS

1. The cold-dry (harmattan) season of the Sahel Savannah zone of Nigeria may not be optimum for work output in the camels studied.

2. Values of RT, RR and HR of camels increased significantly after packing and trekking.

3 TP was significantly higher following packing and trekking in both experimental and control camels.

4. PCV was significantly higher in experimental camels than control camels following packing.

5. The activity of catalase increased significantly following packing in the camels.

6. EOF of experimental camel showed a significant increase in haemolysis after packing and the values obtained were significantly higher than those of control camels.

6.3 RECOMMENDATIONS

i. There is a need to conduct this study during other seasons (the hot-dry and rainy seasons) in the Sahel Savannah zone of Nigeria, since camels are used for work throughout the year.

ii. Camels should be used for packing during the early hours of the harmattan in the Sahel Savannah zone when AT is relatively low.

iii. There is need to investigate the ameliorative effect of antioxidants in camels, subjected to packing during the harmattan season in the Sahel Savannah zone of Nigeria.

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